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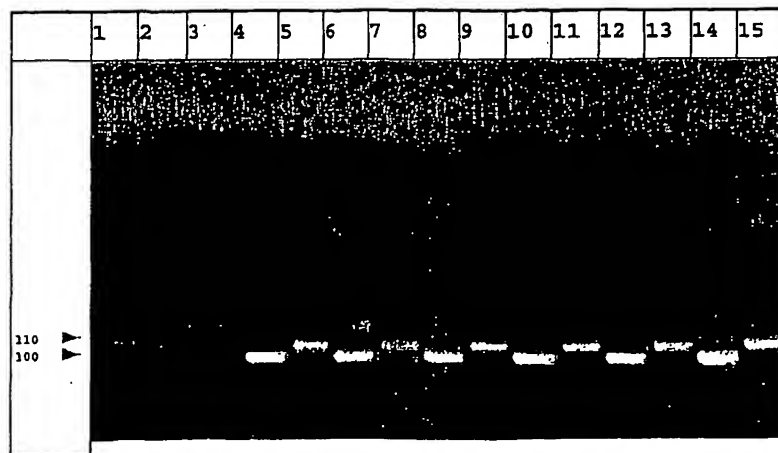
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(54) Title: MYROTHECIUM SP. TRANSFORMATION AND EXPRESSION SYSTEM



(57) Abstract: The present invention is related to a transformation and an expression system in which Myrothecium sp. host cells are used to express homologous or heterologous proteins or are used to genetically engineer metabolic pathways.

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MYROTHECIUM SP. TRANSFORMATION AND EXPRESSION SYSTEMField of the invention

- 10 [0001] The present invention is related to a method to express proteins or to modify protein expression in a microbial host.

Background of the invention

- 15 [0002] Since the advent of the genetic engineering a number of living cells has been transformed with DNA in order to produce homologous or heterologous proteins or to modify their metabolism by introducing for example new metabolic pathways. The variety of cloning hosts spans from
20 bacteria such as *Escherichia coli* till human cells and comprises organisms as diverse as yeasts, fungi or insect cells.

- [0003] Among these organisms, the filamentous fungi have been the subjects of many studies. Indeed some of
25 these organisms are industrially important, such as *Aspergillus* sp., *Trichoderma* sp. or *Fusarium* sp.

- [0004] The first report of a DNA-mediated transformation of a filamentous fungi was published in 1973 using *Neurospora crassa* (Mishra, N.C. and Tatum E.L., 1973.
30 Non-Mendelian inheritance of DNA-induced inositol independence in *Neurospora*. Proc. Nat. Acad. Sci. USA. 70:3875-3879). Later on, a protoplast transformation system has been developed for the same organism (Case et al, 1979. Efficient transformation of *Neurospora crassa* by utilising

hybrid plasmid DNA. Proc. Nat. Acad. Sci. USA. 76:5259-5263). Transformation of *Podospora anserina* (Stahl et al, 1982. Replication and expression of a bacterial-mitochondrial hybrid plasmid in the fungus *Podospora anserina*. Proc. Nat. Acad. Sci. USA. 79:3641-3645) and *Aspergillus nidulans* (Tilburn et al, 1983. Transformation by integration in *Aspergillus nidulans*. Gene. 26:205-221; Ballance et al, 1983. Transformation of *Aspergillus nidulans* by the orotidine-5'-phosphate decarboxylase gene of *Neurospora crassa*. Biochem. Biophys. Res. Comm. 112:284-289) were also reported. The same techniques have been successfully used to transform the commercially important industrial fungi *Aspergillus niger* (Buxton et al, 1985. Transformation of *Aspergillus niger* using the *argB* gene of *Aspergillus nidulans*. Gene. 37:207-214), *Aspergillus oryzae* (Gomi et al, 1987. Agric. Biol. Chem. 51:2549-2555) and *Trichoderma reesei* (Penttillä et al, 1988. Gene. 61:155-164).

[0005] Today transformations systems have been reported for many fungal classes (see for example the chapter on Genetic manipulation of fungi by transformation by Lemke P.A. and Peng M. in The Mycota volume II Genetics and Biotechnology, Kück ed, Springer-Verlag, 1995).

[0006] Some of these are patented (see for example patent EP0184438B1 on "Transforming *Aspergillus* and plasmids for use therein" or patent application WO9602653 on "Thermophilic fungal expression system"). Also are described parts of transformation systems such as the use of promoters or plasmids (For example EP0489718 "Process for the production of protein products in *Aspergillus oryzae* and a promoter for use in *Aspergillus*").

[0007] Enzymes are proteins that are produced by all living organisms. They are highly specific biological catalysts that speed up chemical reactions selectively.

[0008] They are produced industrially for a number

of application ranging from detergent formulation to food application (baking, fruit processing,...) (for reviews see the books of Uhlig, H., Industrial enzymes and their applications, 1998, John Wiley and sons, Inc; Enzymes in food technology, 2002, Whitehurst, R.J. and Law, B.A. eds, 5 Sheffield Academic Press; Industrial enzymology, 1996, Godfrey, T. and West, S. eds., Macmillan Press ltd).

[0009] About 90% of all enzymes used in industrial processing are produced by fermentation of microorganisms.

10 [0010] Today very few species of microorganisms are used for industrial enzyme production. This is a limitation imposed by producing companies needing the widest market range for their products, including food processing.

[0011] However, this situation is changing as new 15 opportunities outside food processing are appearing. Also the increasing introduction of productions using genetically manipulated microorganisms strengthens the need to find new organisms to be used. The genetic engineering allows also the possibility to produce enzymes naturally 20 produced by microorganisms having poor growing properties in fermentors.

[0012] Besides enzyme production, genetically modified microorganisms are used for many other applications such as but not restricted to therapeutical 25 drugs production (antibiotics, anticancer,...), new metabolic pathway expression,...

[0013] Therefore, there is still a need for expression systems that would allow high or modulated levels of production of various compounds.

30 [0014] The diversity of the microbial world is today largely underexploited. It has been estimated that less than 1% of the microorganisms living on earth have already been discovered. Among those identified, very few have been

the subject of investigations other than morphological or taxonomical ones.

[0015] It is therefore the subject of the present invention to present the results of a screening of this microbial diversity and to characterise a microbial genus that would present the characteristics required for a performing cloning host cell: good growth in industrial conditions, transformability, high expression level or well modulated expression level of homologous or heterologous genes or gene sets.

Summary of the invention

[0016] The present invention relates to an isolated *Myrothecium* host cell comprising at least one recombinant DNA construct, or nucleic acid construct, for the modulated expression of homologous genes and/or for the expression of heterologous genes.

[0017] Advantageously, *Myrothecium* host cells were found easy to transform, easy to culture, had a high growth rate coupled to high biomass production and were found to be suitable for growth in fermentors such as for large scale or industrial production of proteins of interest, including but not limited to enzymes such as amylases and xylanases or therapeutic drugs. Applications include but are not limited to protein and/or enzyme production for food and/or therapeutic applications. Another application concerns the use of a genetically engineered (transformed) *Myrothecium* strain as source of biopesticide.

[0018] In an embodiment of the invention, the DNA construct or nucleic acid construct integrates into the host cell chromosome. Alternatively, it may be present on an episome such as a plasmid.

[0019] The DNA construct or nucleic acid construct is built to allow modified expression (such as

overexpression) of a homologous gene and/or may be built to allow expression of a heterologous gene. The protein produced by the transformed *Myrothecium* strain may be a *Myrothecium* protein, a fungal protein as well as a protein normally produced by another organism (i.e. non-fungal). The gene coding for the protein of interest may be engineered and or its codon-use adapted to further increase protein expression or may be engineered to incorporate mutations resulting in altered proteins.

10 [0020] The DNA construct or nucleic acid construct, if needed, comprises at least one operably linked tool that allows or enhances protein expression, said tool being selected from the group consisting of a promoter, a terminator, a polyadenylation signal, a leader, a secretion
15 signal, a selection marker or reporter gene. Said tool may be of heterologous or homologous origin. It falls within the skills of an artisan to define type, multitude and sense of the genes and/or tools in the expression construct that give rise to optimal protein expression.

20 [0021] Preferred selection marker genes are the hygromycin B resistance gene, the phleomycin resistance gene, the phosphinothricin resistance gene, the acetamidase gene, a pyrG gene, an argB gene, a niaD gene and a trpC gene.

25 [0022] A preferred reporter gene is the gene coding for the TAKA-amylase of *Aspergillus oryzae*, optionally associated with flanking regions of the coding region from said TAKA-amylase gene or with flanking regions of the coding region from others genes.

30 [0023] Preferred promoters are the *Aspergillus oryzae* TAKA-amylase promoter, the *Rhizomucor miehei* aspartic proteinase promoter, the *A. niger* glucoamylase promoter, the *A. niger* neutral α -amylase promoter, the *A. niger* acid stable α -amylase promoter, the *R. miehei* lipase

promoter and the promoters of the glycolytic enzymes genes GPD, PGK and ADH.

[0024] Said promoter may be a regulatable/inducible promoter or a constitutive promoter.

5 [0025] Suitable *Myrothecium* sp. include *Myrothecium inundatum*, *Myrothecium prestonii*, *Myrothecium leucotrichum*, *Myrothecium cinctum*, *Myrothecium masonii*, *Myrothecium roridum*, *Myrothecium verrucaria*, *Myrothecium carmichaelii*, *Myrothecium lachastrae*, *Myrothecium atrum*, *Myrothecium*
10 *atroviride*, *Myrothecium gramineum* (syn. *Xepiculopsis graminea*).

[0026] Especially the *Myrothecium gramineum* strain MUCL39210, the *Myrothecium gramineum* strain CBS449.71, the *Myrothecium gramineum* IMI140595, the *Myrothecium gramineum*
15 IMI290405 and the *Myrothecium verrucaria* strain CBS328.52 were found highly suitable hosts for increased protein production, more in particular large-scale or industrial protein production.

[0027] An embodiment of the invention relates to
20 *Myrothecium* cells transformed to comprise a PCNS43 or a p3SR2 vector.

[0028] The invention further relates to the use of the above-described *Myrothecium* transformants. They are very suitable for the use as cell factory, in particular
25 for the production of proteins and/or enzymes. Possible applications include industrial protein and/or enzyme production, and therapeutical drugs production. Another aspect of the inventions concerns the use of *Myrothecium* transformants as source of biopesticide.

30 [0029] Another aspect of the present invention concerns methods of transforming *Myrothecium* sp., said methods comprising the steps of growing *Myrothecium* cells or protoplasts; introducing into said cells or protoplasts at least one recombinant DNA construct for the modulated

expression of homologous genes and/or for the expression of heterologous genes into *Myrothecium* cells; and then selecting transformed i.e. genetically modified *Myrothecium* cells. Suitable transformation techniques include PEG-mediated transformation, electroporation, particle bombardment and/or *Agrobacterium*-mediated transformation. These and other suitable transformation techniques are well known in the art. The DNA construct with which to transform the *Myrothecium* host cells may be a plasmid or a vector.

10 [0030] Another aspect of the invention relates to isolated transformed or genetically modified *Myrothecium* strains obtainable by a method according to claim 18. These transformants or genetically modified strains preferably are characterized by an increased amylase activity, 15 increased xylanase activity, increased growth rate, increased biomass production and/or reduced protease production. Advantageously, the transformants or genetically modified strains will have, after transformation with a suitable DNA construct or nucleic acid construct, an altered metabolic pathway compared to 20 the non-transformed *Myrothecium* strain.

[0031] A further aspect of the present invention concerns a method for producing a protein of interest, said method comprising the steps of culturing transformed or 25 genetically modified *Myrothecium* host cells of the invention under conditions which permit expression of the protein and then recovering the proteins of interest from said *Myrothecium* culture. Said protein preferably is a fungal protein such as a fungal enzyme but may as well be 30 any other protein that can be expressed in *Myrothecium* sp.

[0032] A still further aspect of the present invention concerns a method for producing the biomass of the transformed or genetically modified *Myrothecium* host cells, said method comprising the steps of culturing said

host cells and recovering them with or without the culture medium. Said method thus optionally comprises a purification step to separate *Myrothecium* cells from culture medium or other compounds.

5

Short description of the drawings

[0033] Figure 1 shows a phylogenetic tree constructed with fungal ITS sequences available in the NCBI database or provided in the present invention.

10 [0034] Figure 2 shows a partial nucleotide sequence and its deduced protein sequence of the *Myrothecium gramineum* MUCL39210 glyceraldehyde-3-P dehydrogenase gene

[0035] Figure 3 shows a multiple sequence alignment of four partial GPD protein sequence from the strains
15 MUCL39210, MUCL11831, CBS449.71 and IMI290405.

[0036] Figure 4 shows an agarose gel electrophoresis of the DNA fragments amplified by PCR from *Myrothecium* transformants and corresponding to a part of the *Aspergillus oryzae* α -amylase reporter gene (odd lanes from
20 5 to 13) and to a part of the hygromycin selection gene (even lanes from 4 to 12). Lanes 2-3 and 14-15 correspond respectively to negative and positive controls.

[0037] Figures 5A-E show the 28s rDNA sequence of the strains MUCL39210 (A), MUCL11831 (B), CBS449.71 (C),
25 IMI140595 (D) and IMI290405 (E).

[0038] Figures 6A-E show the ITS sequences of the strains MUCL39210 (A), MUCL11831 (B), CBS449.71 (C), IMI140595 (D) and IMI290405 (E).

[0039] Figures 7A-C show the DNA sequences of the
30 *gpd* gene of the strains MUCL11831 (A), CBS449.71 (B) and IMI290405 (C).

[0040] Figure 8 shows a consensus sequence of the *gpd* gene of the strain MUCL39210.

[0041] Figure 9 shows the amino acid sequence of the glyceraldehyde 3-P dehydrogenase of *Myrothecium gramineum* (*Xepiculopsis graminea*) MUCL39210.

5 Detailed description of the invention

[0042] A first aspect of the invention is the screening of a collection of fungi. The collection screened in the present invention is the collection from the MUCL ("Mycothèque de l'Université Catholique de Louvain", Place
10 Croix du Sud, 2, B-1348 Louvain-la-Neuve, Belgium). However, it will be obvious for a person skilled in the art that other public or private microorganisms collections can be screened following the same approach to provide the selected genus with the required properties. Examples of
15 public collections where fungal strains are available are ATCC (American Type Culture Collection - P.O. Box 1549, Manassas, VA 20108, USA), CBS (Centraalbureau voor Schimmelcultures - P.O. Box 85167, 3508 AD Utrecht, The Netherlands), DSMZ (Deutsche Sammlung von Mikroorganismen
20 und Zellkulturen GmbH - Mascheroder Weg 1 b, D - 38124 Braunschweig, Germany), IMI (International Mycological Institute - Bakeham Lane, Egham, Surrey TW20 9TY, UK),...

[0043] 3.000 different species or strains of fungi were first selected from the MUCL collection based on
25 several criteria such as but not restricted to their non-pathogenicity or the fact that no transformation for this species has been reported in the scientific literature or in a patent. All these strains were grown in liquid culture and on solid medium. Useful liquid media are rich media
30 containing for example glucose, peptone, yeast extract, malt extract, soy meal,... Useful solid media are for example PDA (Potato Dextrose Agar), MA (Malt Extract Agar). The first selection criteria were based on the growth rate, the biomass production, the protein production, the enzyme

production (xylanase, amylase and proteases) and the morphology of the fungal strains.

[0044] The growth rate can be usefully determined for example by measuring the rate of increase of the growth diameter of a single colony on a solid medium.

The biomass production can be usefully determined by evaluating for example the dry matter of cells collected by filtration or by centrifugation at various time intervals during the culture.

10 [0045] The protein production can be usefully determined, using methods well known by persons skilled in the art, by measuring the total amount of proteins in a culture supernatant. Examples of such methods are the Lowry (Lowry O.H. et al. 1951. J. Biol. Chem. 193:265) or the
15 Bradford (Bradford M.M. 1976. Anal. Biochem. 72:248) methods.

[0046] The enzyme production can be usefully determined by measuring the amount of enzyme produced in a culture supernatant using methods well known by persons
20 skilled in the art. Examples of methods of xylanase activity determination are the measure of the release of reducing sugar from xylan (Miller G.L. 1959. Anal. Chem. 31:426) or the measure of the release of coloured compounds from modified substrates (for examples AzowAX or Xylazyme
25 AX from Megazyme). Amylase activity can be determined for example by measuring the rate of release of reducing sugar from starch (Miller, op.cit.) or by using specific commercially available kits (amylase kit from Sigma; Amyl kit from Roche Diagnostics,...). Protease activity can be
30 determined for example using commercially available kits (Azocasein from Megazyme,...).

[0047] The morphology of the fungal strains can be advantageously studied by examining portions of the

cultures with a microscope (light microscope, electronic microscope,...).

[0048] From this screening were retained about one tenth of the 3.000 fungal species or strains. Among the
5 selected strains, about 80 strains were chosen based on their good protein production, about 80 based on their good biomass production, about 60 based on their good amylase production, about 60 based on their good xylanase production and about 10 based on their good or bad protease
10 production.

[0049] In one aspect it may be useful to obtain strains that produce very low levels of proteases. This reduces the risk of proteolytic degradation of proteins of interest possibly produced from introduced heterologous
15 genes. Alternatively, good protease producers might be of interest with the purpose of isolating and using for instance a promoter thereof to enhance protein and/or enzyme production in other strains. In one aspect it may be useful to obtain strains that produce very low levels of
20 proteases. This reduces the risk of proteolytic degradation of proteins of interest possibly produced from introduced heterologous genes. Alternatively, good protease producers might be of interest with the purpose of isolating and using for instance a promoter thereof to enhance protein
25 and/or enzyme production in other strains.

[0050] The selected strains were submitted to a second round of screening in liquid cultures using five different culture media, each one having a particular purpose. The first medium has been selected for maximal protein
30 production, the second for maximal biomass production, the third for the amylase induction, the fourth for xylanase induction and the last one for the protease induction.

[0051] Typical media for protein and/or biomass production are rich media composed for example of carbon

and nitrogen sources such as glucose, malt extract, peptone, soy meal,... A typical medium for amylase production contains usually starch or starch derivatives as enzyme inducer. A typical medium for xylanase production usually
5 contains an hemicellulose-rich inducer such as but not restricted to xylan, wheat straw or wheat bran. A typical medium for protease production is usually enriched in various proteins or amino acids sources.

[0052] The screening criteria retained were the same
10 as for the first step: the growth rate, the biomass production, the protein production, the production of amylase, xylanase and/or protease and the morphology.

[0053] A statistical approach was followed to identify the strains that ranked with a high score for one
15 or several criteria in more than one culture medium. About 50 strains were selected out from this second screening.

[0054] In order to eliminate those strains that would produce any antimicrobial compounds, the production of such compounds by the selected strains has been checked.

20 The inhibition of growth of some bacterial species has been tested using the method described by Färber et al (Färber P. and Geisen R. 1994. Antagonistic activity of the food-related filamentous fungus *Penicillium nalgiovense* by the production of penicillin, Appl. Environ. Microbiol.
25 60:3401-3404). Indicator strains were the following: *Micrococcus flavus* (MUCL collection, determined with API 20E), *Bacillus subtilis* DSM6633, *Bacillus cereus* LMG6923, *Staphylococcus aureus*, *Salmonella* sp. (MUCL collection, determined with API 20E), *Escherichia coli*, *Proteus*
30 *mirabilis* (MUCL collection, determined with API 20E), *Candida blankii* MUCL29808. These strains have been chosen according to Färber et al (op. cit.), to Aissaoui & al (Aissaoui H., Agut M., Torras M. 1999. Effect of the raw extract of *Arthrimum* strains (Hyphomycetes, Dematiaceae)

on the growth of pathogenic bacteria in poultry feed. Microbios. 100(396):109-115) and to the Oxoid Manual (7th edition 1995). It is also obvious for a person skilled in the art that other bacterial species or strains can equally
5 be used for the evaluation.

[0055] Potential producers of antibacterial compounds have been identified and discarded from the list of selected strains.

[0056] A similar method has been used to control the
10 production of antifungal compounds using *Botrytis cinerea* MUCL30834, *Fusarium moniliforme* MULC14280 and *Alternaria alternata* MUCL16089 (Mangiarotti A., Frate G., Picco AM., Caretta G. 1987. Antagonistic activity in vitro of some saprophytic fungi occurring on the phylloplane of rice,
15 wheat and maize. Boletín Micológico. 3:183-189). Again the potential antifungal compounds producers were discarded from the list.

[0057] 30 of the remaining strains (the best ranking in the statistical analysis) were then tested for their
20 ability to incorporate foreign DNA as well as to express a reporter gene. The general method of protoplasts transformation (Punt P.J. and van den Hondel C.A.M.J.J. 1992. Transformation of filamentous fungi based on hygromycin B and phleomycin resistance markers, Methods in
25 Enzymology. 216:447-457) has been followed. For each strain several parameters, that a person skilled in the art will recognise as critical, were investigated. Among these parameters were:

- the possibility to use one or several of the common
30 transformation markers (hygromycine B resistance gene, phleomycin resistance gene, phosphinothricine resistance gene, acetamidase gene, pyrG gene, argB gene, niaD gene, trpC...)

- the conditions to obtain viable protoplasts in large amounts (culture medium, culture temperature, length of the culture, type of digesting enzymes, duration of cell walls digestion, type of osmotic stabiliser,...
- 5 - the conditions of transformation.
- the conditions of protoplasts regeneration.

[0058] A useful reporter gene is the well-known gene coding for the TAKA-amylase of *Aspergillus oryzae*. The flanking regions of the coding region (essentially the promoter and the terminator sequences) or flanking regions of the coding region from others genes are advantageously associated with the coding region to provide a complete expression cassette. The sequence of the TAKA-amylase gene is publicly available (Tada, S., Iimura, Y., Gomi, K., 10 Takahashi, K., Hara, S. and Yoshizawa, K. 1989. Cloning and nucleotide sequence of the genomic Taka-amylase A gene of *Aspergillus oryzae*. Agric. Biol. Chem. 53:593-599) and the corresponding DNA can be easily cloned using for example PCR techniques.

20 [0059] It is therefore the object of the present invention to show that the genus *Myrothecium* presents all the characteristics needed for a performing cloning host such as but not limited to good transformability, good expression of homologous and heterologous genes or genes sets, easy metabolic pathway engineering, good performances 25 in fermentation,

[0060] In a specific aspect of this embodiment, it is more precisely shown that the species *Myrothecium gramineum* (syn. *Xepiculopsis graminea*) presents all the characteristics needed for a performing cloning host such as but not limited to good transformability, good expression of homologous and heterologous genes or genes sets, easy metabolic pathway engineering, good performances 30 in fermentation,

[0061] In a more specific aspect of this embodiment, it is more precisely shown that the strain *Myrothecium gramineum* (syn. *Xepiculopsis graminea*) MUCL39210 presents all the characteristics needed for a performing cloning
5 host such as but not limited to good transformability, good expression of homologous and heterologous genes or genes sets, easy metabolic pathway engineering, good performances in fermentation, ...

[0062] The genus *Myrothecium* is defined in the
10 following way:
"Fructification cupulate, sporodochial or synnematos, formed from closely compacted conidiophores arising from a more or less developed stroma and bearing a mass of slimy green to black spores which becomes hard on drying.
15 Fructification surrounded by differentiated marginal hyphae which may be free or laterally compacted into a plectenchymatous wall. Hyaline or darkened setae sometimes present arising from the basal stroma. Conidiophores hyaline, olivaceous or slightly darkened, macronematous,
20 irregularly and repeatedly branched forming several branches at each node, the ultimate branches bearing the conidiogenous cells (phialides) in whorls. Phialides hyaline or darkened at the apex, occasionally percurrent sometimes with a flared collarete, compacted into a dense
25 parallel layer. Spores unicellular, hyaline or dilute olivaceous, black in mass, slimy" (Tulloch M., 1972, The genus *Myrothecium* Tode ex Fr. Mycological Papers n°130, Commonwealth Mycological Institute, Kew, Surrey, England).

[0063] The genus *Myrothecium* includes i.a. the
30 species *Myrothecium inundatum*, *Myrothecium prestonii*, *Myrothecium leucotrichum*, *Myrothecium cinctum*, *Myrothecium masonii*, *Myrothecium roridum*, *Myrothecium verrucaria*, *Myrothecium carmichaelii*, *Myrothecium lachastrae*,

Myrothecium atrum, *Myrothecium atroviride*, *Myrothecium gramineum*,...

[0064] The species *Myrothecium gramineum* or its synonymous species *Xepiculopsis graminea* has the following description:

"Foliicolous. Conidiomata stromatic, scattered to gregarious, seemingly superficial, discoid to cupulate, excipulate with a dense white fringe of long white setae and sterile elements with coiled apices surrounding a central greenish-black agglutinated conidial mass, 220-450 μm diam., 150-190 μm deep (excluding setae); basal stroma moderately developed, composed of a colourless textura angularis in the lower layers gradually merging with textura prismatica above; excipulum well-developed, of compact and colourless textura prismatica to textura porrecta; marginal hyphae with terminal cells 7-16 x 2-3 μm , of two kinds: those on the inside subcylindrical with blunt apices, olivaceous to fuliginous, irregularly tuberculate, and those on the outside with a subcylindrical, olivaceous to fuliginous, irregularly tuberculate basal part, and a slender, colourless, smooth, sinuate apical process up to 47 μm long and 1 μm wide.

[0065] Conidiomatal setae sparse arising from outer cells of the excipulum, unbranched, straight or slightly incurved, subulate, thick-walled with continuous narrow lumen, smooth-walled, colourless, up to 450 μm long, 15-20 μm wide at the broadest point, up to 1.5 μm wide at the acute apex. Conidiophores arising from the uppermost cells of the basal stroma and inner layer of cells of the excipulum in the concavity of the conidiomata, unbranched or branched, septate, colourless to almost colourless, smooth. Conidiogenous cells discrete, occasionally integrated, subcylindrical to clavate with flared collarettas and moderate to marked apical periclinal

thickenings, more or less pale olivaceous in the upper part with a deeper pigmentation near the collarettes, smooth, (7-)12-16 x 2-3 [mean = 14 x 2.5] μm . Conidia fusiform to ellipsoid with an acute apex and a narrow truncate base, unicellular, pale olivaceous, smooth, 6.5-12.5 x 2-3(-3.5) [mean = 9 x 2.5] μm , with an apical mucoid appendage; mean conidium length/width ratio = 3.6:1. (NAG RAJ T.R. 1993. Coelomycetous anamorphs with appendage-bearing conidia, published by Mycologue Publication)

10 [0066] It should be understood that in the present invention the terms *Myrothecium sp.*, *Myrothecium gramineum* or *Xepiculopsis graminea* refer not only to those organisms present currently under these names in the various public or private microorganisms collections around the world but also to the species which have previously been or currently are assigned to other species but that possess the same morphological and cultural characteristics as defined above.

[0067] The scope of the present invention also includes mutant strains of *Myrothecium sp.*, *Myrothecium gramineum* or *Xepiculopsis graminea*. Types of mutants include but are not restricted to auxotrophic mutants, protease(s) deficient mutants, secretion mutants, constitutive mutants, deregulated mutants. Mutants can be obtained using techniques well known by persons skilled in the art.

[0068] In another embodiment of this invention, it will be referred to rDNA sequences or ITS sequences to assign a particular strain to a species or a genus.

30 [0069] The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) has been widely used to reconstruct microbial phylogeny. The ITS region separating 18s and 26s nrDNA and the 5.8s coding region sequence has been widely used to characterise interspecific and

intergenic levels divergences. The ITS region is therefore useful to gain insight into DNA sequence evolution. This is due to the fact that, as a transcribed, but untranslated sequence, it is free to vary.

5 [0070] Using a combination of the morphological taxonomical methods and the ITS sequences determination and comparison, the inventors have shown that some of the strains deposited as *Myrothecium gramineum* (or as its synonymous *Xepiculopsis graminea*) in public culture
10 collections have been erroneously identified as *Myrothecium gramineum* or *Xepiculopsis graminea*. They have also shown that representative members of other species of the genus *Myrothecium* also present the characteristics needed for a performing cloning host such as but not limited to good
15 transformability, good expression of homologous and heterologous genes or genes sets, easy metabolic pathway engineering, good performances in fermentation,

[0071] Other species of the genus *Myrothecium* are therefore considered as integral part of the present
20 invention.

[0072] *Aspergillus* species and in particular *Aspergillus niger* have been used since years as cell factories to produce homologous and heterologous proteins and can therefore be considered as the models for gene
25 expression in filamentous fungi (see for example the review van Gorcom R.F.M., Punt P.J., van den Hondel C.A.M.J.J. 1994. Heterologous gene expression in *Aspergillus* in The genus *Aspergillus*: from taxonomy and genetics to industrial applications Powell K.A., Renwick, A., Peberdy J.F. eds.
30 Plenum Press, New York, pp 241-250).

[0073] It is therefore one of the objects of the present invention to show that strains from the selected filamentous fungi genus produce, when transformed with

homologous or heterologous DNA more protein and/or enzyme than *Aspergillus niger* in the same conditions.

[0074] It is a further object of the present invention to provide nucleic acid or DNA constructs (recombinant DNA constructs) that, when used to transform *Myrothecium sp.*, *Myrothecium gramineum* or *Xepiculopsis graminea*, allow the expression of the homologous or the heterologous gene present on this nucleic acid or DNA construct. The foreign (introduced) DNA may integrate into the host genome or may be present on an episome. A particular result of the present invention is that the DNA when introduced in the host cell, integrates in the host cell chromosome. This phenomenon is similar to what has already been described for many other species of fungi. Therefore, it is obvious for a person skilled in the art that the same type of constructs as the one used for other species of filamentous fungi can be used for *Myrothecium sp.*, *Myrothecium gramineum* or *Xepiculopsis graminea*. For example, the DNA constructs that are useful in the transformation of *Aspergillus* species such as *Aspergillus niger*, *Aspergillus nidulans* or *Aspergillus oryzae* are also useful for the purpose of the present invention. The promoter may be any DNA sequence that induces a transcriptional activity in the host and may be derived from any homologous or heterologous gene. Examples of promoters are the *Aspergillus oryzae* TAKA-amylase promoter, the *Rhizomucor miehei* aspartic proteinase promoter, the *A. niger* gluocamylase promoter, the *A. niger* neutral α -amylase promoter, the *A. niger* acid stable α -amylase promoter, the *R. miehei* lipase promoter and the promoters of glycolytic enzymes genes such as GPD, PGK, ADH. These promoters may be regulatable/inducible or not. Transcription terminator and polyadenylation sequences may also be added to the nucleic acid or DNA constructs.

[0075] In a particular aspect of this embodiment, it is also provided additional tools to enhance the gene expression in the selected host strains, namely *Myrothecium sp.*, *Myrothecium gramineum*, or *Xepiculopsis graminea*. Using methods well known by persons skilled in the art, the gene coding for the glyceraldehyde 3-phosphate dehydrogenase (GPD gene) has been partially cloned. Flanking regions (promoter and terminator) of these sequences highly suited to make nucleic acid or DNA constructs that give a better expression in the selected host strains.

[0076] In a further aspect of this embodiment, the nucleic acid or DNA constructs may comprise particular sequences, such as sequences coding for a leader or a signal peptide, that allows the protein of interest to be secreted in the extracellular medium. This preregion may be derived from any secreted protein, or may be synthesised using consensus sequences, provided that this preregion allows the secretion of the desired protein.

[0077] In yet another aspect of this embodiment, the nucleic acid or DNA constructs may comprise genes to allow the selection of the transformants of *Myrothecium sp.*, *Myrothecium gramineum* or *Xepiculopsis graminea*. Those selection markers are for example but are not restricted to the acetamidase gene (*amdS*) of *Aspergillus sp.* or the hygromycine resistance gene (*hygB*) of *Escherichia coli*. Other markers include the *argB*, *niaD*, *trpC* or *pyrG* genes of *Aspergillus sp.*

[0078] In yet another aspect of this embodiment, the various elements of the DNA constructs may be either incorporated in the same vector or plasmid or DNA fragment or may be included in separate vectors, plasmids or DNA fragments provided that the DNA sequences required for gene expression are associated with their respective coding sequence.

[0079] An example of suitable selection vector is the plasmid pCSN43 that contains the hph gene of *Escherichia coli* under the control of *Aspergillus* transcriptional signals (Staben C. et al. 1989. Use of a bacterial hygromycin B resistance gene as a dominant selectable marker in *Neurospora crassa* transformation. Fungal Genetics Newsl. 36:79). It confers to transformed or genetically modified cells resistance to hygromycin B.

[0080] Another example of suitable selection vector is the plasmid p3SR2 that contains the acetamidase gene from *Aspergillus nidulans* (Hynes et al. 1983. Isolation of genomic clones containing the amdS gene of *Aspergillus nidulans* and their use in the analysis of structural and regulatory mutations. Mol. Cell. Biol. 3:1430). It confers to transformed or genetically modified cells the ability to grow on a minimal medium containing acetamide as sole nitrogen source.

[0081] In another embodiment of the present invention, a method is provided to transform *Myrothecium* sp., *Myrothecium gramineum* or *Xepiculopsis graminea*. The preferred method of transformation is the standard protoplast transformation. In this method, the DNA constructs are incubated in particular conditions with protoplasts of the host strain. The transformants are first selected on the basis of the selection marker used (for example amdS or hygB) and in a second step cotransformants that have integrated the desired gene are screened.

It is one of the objects of the present invention to show that, when transformed or genetically modified with two separate DNA constructs (containing for example a selection marker and a reporter gene), most of the transformants of *Myrothecium*, *Myrothecium gramineum* or *Xepiculopsis graminea* have integrated simultaneously both DNA constructs.

[0082] Other methods of transformation can usefully replace the protoplasts transformation and are well known by persons skilled in the art. Examples of alternative methods are the electroporation, the transformation by particle bombardment or the transformation by *Agrobacterium* sp.-mediated recombination.

[0083] In another embodiment of the present invention, it is provided *Myrothecium* sp., *Myrothecium gramineum* or *Xepiculopsis graminea* transformed or genetically modified strains that express or overexpress a particular homologous or heterologous gene or a particular set of genes.

[0084] In another embodiment of the present invention, it is provided *Myrothecium* sp. *Myrothecium gramineum* or *Xepiculopsis graminea* genetically engineered strains with one or several altered metabolic pathways.

[0085] In another embodiment of the present invention it is provided culture media to cultivate the *Myrothecium* sp., *Myrothecium gramineum* or *Xepiculopsis graminea* transformed or genetically modified strains. A typical culture media contain carbon (C) sources and/or nitrogen (N) sources and/or salts and/or secondary metabolites and/or miscellaneous compounds. C sources could be glucose, saccharose, ... as well as complex substrates like arabinoxylan, pectin, ... as well as lipids and organic acids. N sources could be amino acids, nitrate, ammonia, ... as well as complex substrates like yeast extract, potato extract, soy extract, ...

[0086] Salts could be chosen among phosphate salts, Magnesium salts, potassium salts, ... Secondary metabolites could be antibiotics, mycotoxins, hormones, vitamins, ... Miscellaneous compound could be defoamers, antifoamers, emulsifiers, ...

[0087] In another embodiment of the present invention it is provided culture techniques to cultivate the *Myrothecium sp.*, *Myrothecium gramineum*, or *Xepiculopsis graminea* or genetically modified strains. Examples of such culture techniques are liquid cultures in erlenmeyer flasks or fermentor, solid state fermentation, airlift fermentation,...

[0088] This invention will be described in further details in the following examples by reference to the enclosed drawings, which are not in any way intended to limit the scope of the invention as claimed.

EXAMPLE 1

Characterisation of the *Myrothecium* strains

1.1. Molecular characterisation of the *Myrothecium* strains

[0089] The genomic DNA of the strains currently assigned as *Myrothecium gramineum* (or of the synonymous strain *Xepiculopsis graminea*) MUCL39210, MUCL11831, CBS449.71, IMI140595 and IMI290405 has been prepared using the Dneasy Plant Mini Kit (Qiagen).

[0090] The DNA coding for RNA ITS (Internal Transcribed Spacer) and 28S regions were analysed by PCR. The oligonucleotides used for the 28S region were: LR0R (5'-ACCCGCTGAACTTAAGC-3', SEQ ID No 1), LR6 (5'-CGCCAGTTCTGCTTACC-3', SEQ ID No 2), LR3R (5'-GTCTTGAAACACGGACC-3', SEQ ID No 3) and LR3 (5'-CCGTGTTTCAAGACGGG-3', SEQ ID No 4). The oligonucleotides used for the ITS region were ITS1 (5'-TCCGTAGGTGAACCTGCGG-3', SEQ ID No 5) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3', SEQ ID No 6).

[0091] PCR reactions were performed in 0.25 ml tubes, using a MJ Research PTC-200 Peltier Thermal Cycler. PCR reactions (50 µl) contained approximately 20 ng of

5 fungal genomic DNA (in 10 μ l), 10 pM ITS1 and ITS4 primer pairs (1 ml), 100 mM dNTPs (1 μ l), 1X reaction buffer (10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl₂, 50 mM KCl, 0.1 ng/ml BSA) (5 μ l), and 2.5 U Taq polymerase (Invitrogen) (0.5 μ l). Thermal cycling conditions were as follows: for 28S: initial denaturation (94°C, 5 min), followed by 35 cycles of denaturation (94°C, 1 min), annealing (50°C, 1 min 30 s), primer extension (72°C, 4 min), and one final cycle of primer extension (72°C, 10 min); for ITS: initial
10 denaturation (94°C, 3 min), followed by 30 cycles of denaturation (94°C, 1 min 30 s), annealing (55°C, 1 min 30 s), primer extension (72°C, 2 min), and one final cycle of primer extension (72°C, 10 min).

[0092] The reaction products (10 μ l) were analysed
15 by electrophoresis on a 0.8 % agarose gel in Tris-acetate EDTA buffer (TAE buffer) to check the presence of a unique amplified DNA fragment. The PCR product was then purified using PCR purification kit (Qiagen) and quantified with a Biophotometer (Eppendorf).

20 [0093] DNA sequencing reactions were performed in 0.25 ml tubes, using a MJ Research PTC-200 Peltier Thermal Cycler. PCR reactions (20 μ l) contained 25 to 100 fmol of purified PCR product (0.5 to 10 μ l), 1.6 μ M of -47 sequencing primer (2 μ l), 8 μ l of DTCS Quick Start Master
25 Mix (100 μ l of dNTPs, 200 μ l of ddATP, 200 μ l of ddGTP, 200 μ l of ddCTP, 200 μ l of ddUTP, 200 μ l of 10X reaction buffer and 100 μ l Taq polymerase). Thermal cycling conditions were as follows: 30 cycles of denaturation (96°C, 20 sec), annealing (50°C, 20 sec), primer extension
30 (60°C, 4 min), followed by holding at 4°C. The reaction is stopped by adding 4 μ l of Stop Solution (1.5M NaOAc + 50 mM EDTA prepared fresh daily by mixing equal volumes of 3M NaOAc and 100mM EDTA) and 1 μ l of 20 mg/ml glycogen. The sequencing reactions were transferred to a 96 wells plate.

60 μ l of 95% cold ethanol from -20°C freezer were added, the plate was sealed with adhesive foil, mixed thoroughly, incubated at -20°C during 10 minutes, centrifuged at 14.000 rpm at 4°C during 30 minutes and the supernatant was
5 carefully removed. The pellets were then rinsed two times with 200 μ l of 70% cold ethanol. Finally, the samples in the plate were dried under vacuum until no traces of ethanol remained (5 to 15 minutes). The samples were resuspended in 40 μ l of Sample Loading Solution, left at
10 room temperature for ten minutes then mixed thoroughly and overlaid with one drop of light mineral oil (Sigma Cat# M 5904).

[0094] The sequencing was done in a capillary electrophoresis sequencer "CEQ™ 2000 XL DNA Analysis
15 System" (Beckman Coulter). The sequences generated were rectified and aligned with the software Sequencher™ 4.1. The sequence of 28s rDNA of the strain MUCL39210 is shown in SEQ ID No 7.

[0095] The sequence of 28s rDNA of the strain
20 MUCL11831 is shown in SEQ ID No 8.

[0096] The sequence of 28s rDNA of the strain CBS449.71 is shown in SEQ ID No 9.

[0097] The sequence of 28s rDNA of the strain IMI140595 is shown in SEQ ID No 10.

25 [0098] The sequence of 28s rDNA of the strain IMI290405 is shown in SEQ ID No 11.

[0099] The ITS sequence of the strain MUCL39210 is shown in SEQ ID No 12.

[0100] The ITS sequence of the strain MUCL11831 is
30 shown in SEQ ID No 13.

[0101] The ITS sequence of the strain CBS449.71 is shown in SEQ ID No 14.

[0102] The ITS sequence of the strain IMI140595 is shown in SEQ ID No 15.

[0103] The ITS sequence of the strain IMI290405 is shown in SEQ ID No 16.

[0104] These sequences were compared and compared with other closely related sequences of the NCBI gene bank
5 (<http://www.ncbi.nlm.nih.gov/BLAST/>). The sequences were first aligned using the software ClustalX 1.5b and then a bootstrap tree was constructed using the software PAUP 4.0b10.

[0105] A phylogenetic tree with relevant data is
10 presented on figure 1. It is concluded from this tree that some of the investigated *Myrothecium gramineum* (syn. *Xepiculopsis graminea*) strains have been erroneously assigned to a particular species. However, all the strains studied, with the exception of MUCL11831 belong to the
15 *Myrothecium* genus.

1.2. Morphological identification of the *Myrothecium* strains.

[0106] A taxonomical reidentification of the
20 MUCL39210 strain has confirmed that it presents all the morphological characteristics of the species *Myrothecium gramineum* (syn. *Xepiculopsis graminea*) (see the description in the detailed description of the invention).

[0107] A similar examination of the strain MUCL11831
25 confirmed that this strain does not belong to the *Myrothecium* genus.

EXAMPLE 2

Transformation of *Myrothecium*

30 2.1. Choice of selectable markers

[0108] *Myrothecium gramineum* (syn. *Xepiculopsis graminea*) MUCL39210 was incubated on PDA (Potato Dextrose Agar), AMMA (*Aspergillus* Minimal Medium Agar) or MA2 (Malt

Extract Agar) plates with increasing concentration of hygromycin B, phleomycin or phosphinothricine. The threshold antibiotic concentration where no growth occurred was 500 µg/ml for hygromycin B, >100 µg/ml for phleomycin and > 400 µg/ml for phosphinothricin.

[0109] *Myrothecium gramineum* (syn. *Xepiculopsis graminea*) MUCL39210 was incubated on minimal medium plates containing acetamide as sole nitrogen source. The strain did not grow on this medium.

10 [0110] Similar results were obtained with the strains of *Myrothecium gramineum* CBS449.71, IMI140595, IMI290405 and *Myrothecium verrucaria* CBS328.52

2.2. Vectors

15 2.2.a. Selectable vector

[0111] The vector chosen to allow the selection of the transformants by their resistance to an antibiotic is the plasmid pCSN43 that contains the hph gene of *Escherichia coli* under the control of *Aspergillus* transcriptional signals (Staben C. et al. 1989. Use of a bacterial hygromycin B resistance gene as a dominant selectable marker in *Neurospora crassa* transformation. Fungal Genetics Newsl. 36:79).

25 [0112] The vector chosen to allow the selection of the transformed or genetically modified cells by their ability to grow on a minimal medium containing acetamide as sole nitrogen source is the plasmid p3SR2 (Hynes et al. 1983. Isolation of genomic clones containing the amdS gene of *Aspergillus nidulans* and their use in the analysis of structural and regulatory mutations. Mol. Cell. Biol., 3:1430)

2.2.b. Expression vectors

2.2.b.1. p2G-S

[0113] A DNA fragment covering the coding region as well as its terminator region was amplified by a PCR reaction. The synthetic oligonucleotide AMY1 (5'-GGAATTCCACAGAAGGCATTTATG-3', SEQ ID No 17) was chosen to contain the ATG codon of the first amylase methionine. The synthetic oligonucleotide AMY2 (5'-GCTCTAGAGCAACCACCAGGTCA-3', SEQ ID No 18) corresponds to the sequence located about 400 bp downstream of the amylase terminal codon.

[0114] The two oligonucleotides have sequences recognised respectively by the restriction enzymes *EcoRI* and *XbaI*. Both primers (40 pmoles) were used for a PCR reaction with the gDNA from *Aspergillus oryzae* MUCL14492.

The PCR reaction contained 2.5 U *Pfu* DNA polymerase (Stratagene), 20 mM Tris-HCl pH 8.0, 10 mM KCl, 2 mM MgCl₂, 6 mM (NH₄)₂SO₄, 0.1% Triton X-100, 1 µg BSA into a final volume of 100 µl. After denaturation of the DNA during 4 min at 94°C, the reaction was performed with 20 cycles of 30 s at 94°C, 30 s at 55°C and 60 s at 72°C followed by an elongation step of 7 min at 72°C. The amplified DNA fragments were purified in a final volume of 50 µl with the QIAquick PCR purification kit (Qiagen) according to the manufacturer's protocol. The purified fragment was treated with the *EcoRI* and *XbaI* restriction enzymes. The reaction was performed with 5 U *Xba I* and 5 U *EcoR I* (Pharmacia), 1 x One-Phor-All buffer PLUS in 60 µl at 37°C overnight. The restricted fragments were purified with the QIAquick gel extraction kit (Qiagen) after electrophoresis on an agarose gel and eluted into 30 µl water.

[0115] The PCR fragment was inserted between the *EcoR I* and *Xba I* sites of the pBluescript II SK(-) vector (Stratagene). The vector was prepared as follows: 0.5 µg

pBluescript II SK was treated by 5 U *EcoR* I and 5 U *Xba* I restriction enzymes (Pharmacia) in a 20 μ l final volume, 2 x One-Phor-All buffer PLUS at 37°C overnight. After electrophoresis in an agarose gel, it was purified with
5 QIAquick gel extraction kit (Qiagen) and eluted in 30 μ l water. 2 μ l of the PCR fragment was ligated to this vector (1 μ l) in the presence of 1 mM ATP, 1U T4 DNA ligase (Pharmacia) and 1 x One-Phor-All buffer PLUS in a 10 μ l final volume at 16°C overnight. The ligation product (1 μ l)
10 was electroporated into competent DH10b cells (BRL-Gibco) after dialysis against water. The new plasmid was termed pAMY3.

[0116] The promoter of the glyceraldehyde-3-P dehydrogenase gene from *Aspergillus nidulans* was inserted
15 in front of the amylase gene. This promoter allows a rather strong constitutive transcription of genes located downstream of it (Punt, P.J., Dingemanse, M.A., Kuyvenhoven, A., Soede, R.D.M., Pouwels, P.H. and van den Hondel; C.A.M.J.J., 1990. Functional elements in the
20 promoter region of the *Aspergillus nidulans* *gpdA* gene coding for the glyceraldehyde-3-P dehydrogenase. Gene. 93: 101-109 ; Punt, P.J., Zegers, N.D., Busscher, M., Pouwels, P.H. and van den Hondel; C.A.M.J.J., 1991. Intracellular and extracellular production of proteins in *Aspergillus*
25 under the control of expression signals of the highly expressed *Aspergillus nidulans* *gpdA* gene. J. Biotechnol. 17: 19-34). To insert the amylase gene downstream of the GPD promoter, the plasmid pFGPDGLAT2 was digested with the restriction enzyme *Nco*I . After purification with the
30 QIAquick gel extraction kit (Qiagen), the plasmid was blunted with T4 DNA polymerase and dNTP, purified, and restricted with *Xba*I restriction enzyme (5U) in the presence of 1 x One-Phor-All buffer PLUS in a 20 μ l final volume 37°C overnight). The same protocol was applied on

pAMY3 except that the *NcoI* restriction enzyme was replaced by *EcoRI*. The fragments of interest were isolated after agarose gel electrophoresis, purified with QIAquick gel extraction kit (Qiagen) and collected separately in 30 μ l water. The purified promoter and vector DNA fragment (1 μ l) was ligated with the amylase gene and its terminator from pAMY3 (1 μ l) in the presence of 1 mM ATP, 1U T4 DNA ligase (Pharmacia) and 1 x One-Phor-All buffer PLUS in a 10 μ l final volume at 16°C overnight. The ligation product (1 μ l) was electroporated into competent DH10b cells (BRL-Gibco) after dialysis against water. The new plasmid was named pGPD-AMY.

[0117] The GPD promoter, the amylase gene and its terminator have been isolated from pGPD-AMY and inserted again into a new pGPD-AMY in order to have two copies of the functional gene on one plasmid. The first step was to digest pGPD-AMY with 1U *HindIII* restriction enzyme and 1 x One-Phor-All buffer PLUS in a 40 μ l final volume at 37°C overnight. After purification with QIAquick gel extraction kit (Qiagen), the linear DNA fragment was blunted in the presence of T4 DNA polymerase and dNTP. The plasmid was again purified with QIAquick gel extraction kit (Qiagen) and treated with *NotI* restriction enzyme and 1 x One-Phor-All buffer PLUS in a 40 μ l final volume at 37°C overnight. The fragment containing the functional amylase gene was finally isolated on agarose gel after electrophoresis. The pGPD-AMY plasmid (1 μ g) was digested by *XbaI* (1U) and 1 x One-Phor-All buffer PLUS in a 40 μ l final volume at 37°C overnight. After purification with QIAquick gel extraction kit (Qiagen), the linear DNA fragment was blunted in the presence of T4 DNA polymerase and dNTP. The plasmid was again purified with QIAquick gel extraction kit (Qiagen) and digested with the *NotI* restriction enzyme and 1 x One-Phor-All buffer PLUS in a 40 μ l final volume at 37°C

overnight. The vector with the DNA fragment containing a functional amylase gene was isolated after agarose gel electrophoresis. Both fragments were ligated together in the presence of 1 mM ATP, 1U T4 DNA ligase (Pharmacia) and 1 x One-Phor-All buffer PLUS in a 10 μ l final volume at 16°C overnight. The ligation product (1 μ l) was electroporated into competent DH10b cells (BRL-Gibco) after dialysis against water. The new plasmid was termed p2GPD-AMY.

10 [0118] Finally a selection marker was inserted in p2GPD-AMY. The acetamidase gene will allow the growth of fungi on a minimal medium with acetamide as unique nitrogen source. The functional acetamidase gene from *A. nidulans* (AmdS) has been isolated from 1 μ g of p3SR2 plasmid 15 digested with the restriction enzymes *Sal*I (1U) and *Eco*RI (1U) and 2 x One-Phor-All buffer PLUS in a 40 μ l final volume at 37°C overnight. This DNA fragment was inserted between the *Sal*I and *Eco*RI restriction sites of pBluescript II SK. This new plasmid as well as p2GPD-AMY were digested 20 with *Sal*I (or *Xba* I for p2GPD-AMY), blunted by treatment with the T4 DNA polymerase and digested with *Not*I. The functional AmdS gene DNA fragment and the open p2GPD-AMY plasmid were isolated from agarose after gel electrophoresis. Both fragments were then ligated together 25 in the presence of 1 mM ATP, 1U T4 DNA ligase (Pharmacia) and 1 x One-Phor-All buffer PLUS in a 10 μ l final volume at 16°C overnight. The ligation product (1 μ l) was electroporated into competent DH10b cells (BRL-Gibco) after dialysis against water. The new plasmid was termed p2G-S.

2.2.b.2. Cloning of the GPD promoter

Design of the oligonucleotides

[0119] Based on published sequences of fungal glyceraldehyde-3-P dehydrogenases (gpd), a set of four oligonucleotides has been designed in order to amplify a portion of the gpd gene of *Myrothecium*.

These oligonucleotides have the following sequences:

Gpd1 : 5'-GGNATCAAYGGITTCGG-3', SEQ ID No 19

Gpd2 : 5'-GTGSWGSWGGGGATGATGTT-3', SEQ ID NO 20

10 Gpd3 : 5'-GGTCGTATCGTNTTYCGIAAYGC-3', SEQ ID No 21

Gpd4 : 5'-GGAGCCAGGCAGTTGGTIGTRCA-3', SEQ ID No 22

Where N stands for A, T, C or G; Y stands for C or T; I stands for dInosine; S stands for G or C; W stands for A or T; R stands for A or G.

15

Preparation of the genomic DNA

[0120] The genomic DNA of the following strains has been prepared using the Dneasy Plant Mini Kit (Qiagen): MUCL39210, MUCL11831, CBS449.71, IMI140595 and IMI290405.

20

PCR

[0121] PCR reactions have been performed in 0.25 ml tubes with a MJ research PTC-200 Peltier Thermal Cycler. The reaction mix (25 μ l) contained about 20 ng genomic DNA, 10 pM oligonucleotides, dNTP 200 μ M, 1 x buffer (Tris-HCl 10 mM, pH 8.3, MgCl₂ 1.5 mM, KCl 50 mM, BSA 0.1 ng/ml) and 1 unit of Taq polymerase (Invitrogen). Amplification conditions were the following: initial denaturation (94°C, 4 min); 25 cycles of denaturation (94°C, 30 sec)-hybridisation (52°C, 30 sec)-elongation (72°C, 1 min); final elongation (72°C, 7 min).

30

[0122] Cloning of the gpd gene of the strains MUCL11831, CBS449.71 and IMI290405.

[0123] DNA fragments amplified using the protocol described above (with the oligonucleotides gpd1 and gpd2 as amplification primers) have been separated by agarose gel electrophoresis and purified with the aid of a QIAquick Gel Extraction Kit (Qiagen). A second round of PCR has been performed on these purified fragments and the resulting DNA fragments have been directly ligated into the plasmid commercially available pT-Adv (Advantage PCR cloning Kit - Clontech) using the recommendations of the manufacturer. Ligations products have been used to transform competent cells of *Escherichia coli* DH5 α (Invitrogen). Positive clones have been identified by plasmid isolation and analysis by restriction enzyme digestion and agarose gel electrophoresis.

[0124] Sequencing of the gpd genes of the strains MUCL11831, CBS449.71 and IMI290405.

[0125] The insert of the plasmid described above has been sequenced using usual procedures.

[0126] The sequence of the gpd gene of the strain MUCL11831 is shown in SEQ ID No 23.

[0127] The sequence of the gpd gene of the strain CBS449.71 is shown in SEQ ID No 24.

[0128] The sequence of the gpd gene of the strain IMI290405 is shown in SEQ ID No 25.

25

Cloning of the gpd gene of MUCL39210

[0129] The same procedure as for the cloning of the gpd genes of the strains MUCL11831, CBS449.71 and IMI290405, except that the first PCR was performed using the oligonucleotides gpd2 and gpd3 and that the second PCR was performed with the oligonucleotides gpd3 and gpd4.

Sequencing of the gpd gene of the strain MUCL39210.

[0130] The inserts of 8 different inserts of the plasmids obtained above have been sequenced using usual procedure.

- 5 [0131] A consensus sequence of the gpd gene of the strain MUCL39210 is shown in SEQ ID No 26.

Results

- [0132] The MUCL39210 GPD partial sequence shown in
10 SEQ ID NO14 was translated into a putative protein sequence as presented in Figure 2. The GPD partial sequence comprises a large putative intron. The intron sequence does not show homology to any protein from protein databases when translated from the six possible DNA frames. The
15 putative protein sequence (SEQ ID No 27) shows the highest similarity with the GPD protein sequence of *Neurospora crassa* using the BLASTP 2.2.4. program against the non-redundant GenBank database as of 20th Augustus 2002. Between the various *Myrothecium* GPD protein sequences, the
20 identity is 88% as shown in figure 3.

2.3. Transformation2.3.a. Transformation of Myrothecium

- [0133] The strains *Myrothecium gramineum* (syn.
25 *Xepiculopsis graminea*) MUCL39210, *Myrothecium gramineum* CBS449.71, IMI140595, IMI290405, and *Myrothecium verrucaria* CBS328.52 were transformed by generating protoplasts according to the protocol described by Punt et al. (Punt et al. 1992. Transformation of filamentous fungi based on
30 hygromycin B and phleomycin resistance markers. Meth. Enzymol. 216:447).

- [0134] The strains were grown in 350 ml Rich Liquid medium (malt extract 2%, peptone 1% and glucose 3%) at 25°C

for a period between 16 and 36 hours depending on the strain. The culture was filtered through a Miracloth filter to collect the mycelium. The mycelium was washed with the OSM solution (CaCl_2 0.27 M, NaCl 0.6 M) and then incubated
5 with 20 ml OSM solution/g mycelium supplemented with 12 mg/ml β -D-glucanase (Interspex), 7.5 mg/ml driselase (Interspex) and 81.4 U/ml yeast lytic enzyme (ICN). The incubation is done at 25°C with slow agitation (80 rpm).

[0135] The apparition of the protoplasts is followed
10 visually under the microscope and varies from 90 to 180 min, depending on the strain. Once the protoplasts were formed, the suspension was putted on ice. The protoplasts were separated from intact mycelium by filtration through a sterile Miracloth filter and diluted with 1 volume STC1700
15 solution (sorbitol 1.2 M, Tris-HCl pH 7.5 10 mM, CaCl_2 50 mM, NaCl 35 mM) or GTC (glucose 1 M, CaCl_2 50 mM, Tris 10 mM pH8). The protoplasts were then collected by centrifugation at 2000 rpm, 10 min, 4°C and washed twice with STC1700 or GTC solution. They were finally resuspended
20 in 100 μl of STC1700 or GTC (10^8 protoplasts/ml).

[0136] To obtain transformants resistant to hygromycine, c.a. 2 μg pCSN43 plasmid DNA and c.a. 8 μg p2G-S plasmid DNA were added to the protoplasts suspension. To obtain transformants able to grow on acetamide as sole
25 nitrogen source 3 μg p3SR2 plasmid DNA and 9 μg p2G-S plasmid DNA were added to the protoplasts suspension.

[0137] After 30 min at 20°C, 250, 250 and 850 μl PEG solution (PEG 4000 60%, Tris-HCl 10 mM pH 7.5 and CaCl_2 50 mM) were added successively and the suspension was further
30 incubated for 20 min at 20°C. PEG treated protoplasts suspension was diluted by the addition of 50 ml STC1700 or GTC and centrifuged 5 min at 4°C, 2000 rpm. The protoplasts

were then resuspended in 200 μ l STC 1700 or GTC and plated onto selective medium.

[0138] For hygromycin selection Malt Extract Agar Medium or Potato Dextrose Agar osmotically stabilised with 1.2 M sorbitol or 1 M glucose and supplemented with 500 μ g/ml hygromycin was used. In some cases, protoplasts are plated onto the same medium without hygromycin. After 2 hours incubation, hygromycin (500 μ g/l) overlays (8 ml molten agar) were dispensed over the protoplasts.

[0139] For acetamidase selection the AMM medium (Pontecorvo, G., Roper, J.A., Hemmons, L.M., Mc Donald, K.D. and Bufton, A.W.J., 1953. Adv. Genet. 5: 142-152.) supplemented with sorbitol 1.2M and acetamide as sole nitrogen source was used.

[0140] A series of transformants has been obtained for each strain and for each transformation trial. These transformants have been replicated on the same selection medium without sorbitol and kept for further analysis.

2.3.b. Control transformation.

[0141] The same protocol as described in 2.3.a. has been used to transform the MUCL28817 strain of *Aspergillus niger*. A number of transformants have been selected and kept for further analysis.

25

EXAMPLE 3.

Analysis of the transformants

3.1. Amplification of integrated DNA

3.1.a. Preparation of genomic DNA from fungal strains

[0142] The genomic DNA of the transformed or genetically modified fungal strains as well as the control strains has been prepared using the Dneasy Plant Mini Kit (Qiagen)

3.1.b. PCR reactions

[0143] According to the published sequence of the *E. coli* hygromycin resistance gene (*hph* gene), the oligonucleotides *hphpCSN431* (5'-ATGCCTGAACTCACCGCGACG-3',
5 SEQ ID No 28) and *hphpCSN432* (5'-CTATTCCTTTGCCCTCGG-3', SEQ ID No 29) were designed to amplify a region of 1020 bp comprising the *hph* gene.

[0144] To detect the presence of p2G-S into the *Myrothecium* genome without amplification of the endogenous
10 amylase gene or the GPD promoter, the GPD2 (5'-TCTGGCATGCGGAGAG-3', SEQ ID No 30) and AMY5 (5'-CGATGATGCCCTGCCA-3', SEQ ID No 31) oligonucleotides were designed to allow the amplification of a 1134 bp length fragment covering a part of the *A. niger* GPD promoter fused
15 to a part of the sequence of the TAKA amylase gene.

[0145] PCR reactions have been performed in 0.25 ml tubes with a MJ research PTC-200 Peltier Thermal Cycler. The reaction mix (25 μ l) contained about 20 ng genomic DNA, 10 pM oligonucleotides, dNTP 200 μ M, 1 x buffer (Tris-HCl
20 10 mM, pH 8.3, MgCl₂ 1.5 mM, KCl 50 mM, BSA 0.1 ng/ml) and 1 unit of Taq polymerase (Invitrogen). Amplification conditions were the following: initial denaturation (94°C, 4 min); 25 cycles of denaturation (94°C, 30 sec)-hybridisation (55°C, 30 sec)-elongation (72°C, 1 min);
25 final elongation (72°C, 7 min).

3.1.c. Results

[0146] The results of the analysis of 5 different transformants of *Myrothecium gramineum* (syn. *Xepiculopsis graminea*) MUCL39210 selected for the hygromycin resistance
30 and amylase production are shown on figure 4. The agarose gel electrophoresis of the PCR products shows that all the five transformants studied have integrated both the selection marker gene (*hph* gene - c.a. 1000 bp DNA fragment

- lanes 4, 6, 8, 10 & 12) and the reporter gene (α -amylase gene of *Aspergillus oryzae* - c.a. 1100 bp DNA fragment - lanes 5, 7, 9, 11 & 13). Negative controls are presented in the lanes 2 & 3 (respectively products of hygromycine and α -amylase genes PCR amplification). Lanes 14 and 15 are positive controls (PCR reactions performed respectively on the pCSN43 and p2G-S plasmids).

3.2. Evaluation of recombinant protein production

3.2.a. Culture of the transformants

[0147] Twelve transformants of *Myrothecium gramineum* (syn. *Xepiculopsis graminea*) MUCL39210 obtained by transformation with the plasmids pCSN43 and p2G-S as well as 49 transformants of *Aspergillus niger* MUCL28817 have been cultivated in AMM medium with 3% sucrose as carbon source in shaking incubators (140 rpm) at 25°C. The culture volume was 20 ml for a total erlenmeyer volume of 100 ml. After 4 days, the supernatants of the cultures were collected by centrifugation.

20

3.2.b. α -amylase activity determination

[0148] The α -amylase activity was determined by a modification of the method included in the Sigma Amylase kit. The substrate was diluted in a 100 mM NaH_2PO_4 buffer pH 6.0. One amylase unit was defined as the amount of enzyme that gives an absorbance of 1 OD unit at 405 nm after 15 min reaction at 37°C and when 120 μl of the reaction mix are read in a 96 holes microplate with the model 550 microplate reader (Bio-Rad).

25

3.2.c. Biomass determination (Dry matter determination)

[0149] The amount of biomass in a culture was determined by filtering an aliquot of the culture through a

30

Whatman n°540 filter and putting the filter in an oven at 105°C for 48 hours.

3.2.d. Results

5 Table 1

	MUCL39210		MUCL28817	
	U/ml	U/g biomass	U/ml	U/g biomass
WT	1,33	92,5	0,45	27,5
Tf. 1	5,75	428	20,18	1127,5
Tf. 2	44,5	3888	1,225	75
Tf. 3	18,5	1488	26,03	1355
Tf. 4	11,9	933	21,53	1145
Tf. 5	20,8	1675	25,13	1325
Tf. 6	21,5	1683	5,925	345
Tf. 7	32,5	2348	24,53	1270
Tf. 8	34,3	2718	7,95	405
Tf. 9	59,5	4035	5,55	307,5
Tf. 10	32,5	2850	22,05	1122,5
Tf. 11	18,4	1430	2,3	142,5
Tf. 12	46	3680	9,85	497,5
Tf. 13			20,3	1127,5
Tf. 14			25,35	1302,5
Tf. 15			2,025	110
Tf. 16			2,1	122,5
Tf. 17			5,35	302,5
Tf. 18			21,63	1205
Tf. 19			2,725	152,5
Tf. 20			25,35	1282,5
Tf. 21			20,38	1032,5
Tf. 22			22,18	1027,5
Tf. 23			1,95	120
Tf. 24			11,05	577,5
Tf. 25			2,95	177,5
Tf. 26			13,2	667,5
Tf. 27			0,825	52,5
Tf. 28			9,675	540
Tf. 29			28,65	1552,5

Tf. 30			1,775	110
Tf. 31			26,18	1422,5
Tf. 32			2,925	200
Tf. 33			3,9	225
Tf. 34			1,55	90
Tf. 35			1,2	82,5
Tf. 36			2,275	157,5
Tf. 37			9,425	560
Tf. 38			1,075	62,5
Tf. 39			4,275	277,5
Tf. 40			25,05	1370
Tf. 41			8,175	462,5
Tf. 42			1,7	110
Tf. 43			1,075	70
Tf. 44			1,15	70
Tf. 45			2,275	147,5
Tf. 46			1,525	105
Tf. 47			1,475	90
Tf. 48			27,75	1420
Tf. 49			0,75	47,5
Average	28,8	2263	10,48	562,5

[0150] The table 1 shows that all transformants (Tf.) obtained with the strain *Myrothecium gramineum* (syn. *Xepiculopsis graminea*) MUCL39210 produce significantly more α -amylase than a non-transformed control strain. Many of the *Aspergillus niger* MUCL28817 transformants produce very few α -amylase compared to the non-transformed strain indicating that in those cases, they should not have been transformed by the reported gene or that this reporter gene is integrated under a non-expressible form.

[0151] The *Myrothecium* transformants produce more α -amylase than the *Aspergillus* transformants obtained and cultivated in the same conditions. The best *Xepiculopsis* transformant (n°9) produces more than twice α -amylase compared to the best *Aspergillus* transformant (n°29). On an

average the *Myrothecium* transformants produce 2.75 more α -amylase than the *Aspergillus* transformants.

[0152] Furthermore, the *Myrothecium* transformants have a better specific production of α -amylase (amount of enzyme / g of biomass) compared to the *Aspergillus* transformants.

3.4. Stability of the transformants

[0153] A hygromycin-resistant transformant of *Myrothecium gramineum* (syn. *Xepiculopsis graminea*) MUCL39210 was stored at -80°C . During the storage, the same transformant was successively replicated 20 times on solid PDA (Potato Dextrose Agar) medium without selective pressure. After these replica platings, the two strains (the original transformant and the replicated strain) together with the non-transformed *Myrothecium gramineum* (syn. *Xepiculopsis graminea*) MUCL39210 were grown (24°C - 145 rpm) in 20 ml of liquid medium (AMM medium supplemented with 3% sucrose and 0.5% yeast extract, pH 6.3) in 100 ml erlenmeyers. Samples of the cultures were taken at various times and assayed for α -amylase activity.

Table 2

	Activity U/ml	
	120 HF	144 HF
Wild-type	3,225	9,4
Original transformant	15,9	19,575
Transformant replicated 20 times	16,925	21,375

[0154] As shown in the table 2, the number of replications performed with the *Myrothecium gramineum* (syn. *Xepiculopsis graminea*) MUCL39210 transformant does not affect the productivity of the strain.

3.5. Analysis of transformants of other *Myrothecium* strains

[0155] Similar results (enzymatic activity determination, control of the integration of the DNA constructs) as the ones obtained for transformants of *Myrothecium gramineum* (*Xepiculopsis graminea*) MUCL39210 have been obtained with transformants of other representative strains of the species *Myrothecium gramineum* as well as transformants of strains representative of the genus *Myrothecium*, i.e. *Myrothecium gramineum* IMI140595, *Myrothecium gramineum* CBS449.71, and *Myrothecium verrucaria* CBS328.52. According to the ribosomal DNA sequencing results, it appears that *Myrothecium gramineum* CBS449.71 could belong to another species than *gramineum*.

[0156] The same types of DNA constructs as for *Myrothecium gramineum* (*Xepiculopsis graminea*) MUCL39210 have been used for the transformation of these strains. The transformants obtained have been evaluated as described in example 3.2.

[0157] The strains CBS449.71, IMI140595 and CBS328.52, and their transformants were grown (30°C - 145 rpm) in 70 ml of liquid medium (AMM medium supplemented with 3% sucrose and 0.5% yeast extract, pH 6.3) in 250 ml Erlenmeyer.

[0158] The amylase activity of the strains was measured after 123 hours fermentation. The results of amylase production are presented in table 3.

Table 3

strain		Relative amylase ₁ activity
CBS449.71	wt.	1
CBS449.71	transformant n°13	3.44
CBS449.71	transformant n°2	4.23
CBS449.71	transformant n°45	4.23
CBS449.71	transformant n°1	4.97
IMI140595	wt	1
IMI140595	transformant n°22.1	1.86
IMI140595	transformant n°34.1	2.03
IMI140595	transformant n°1.1	13.81
CBS328.52	wt	1
CBS328.52	transformant n°13	1.47
CBS328.52	transformant n°31	5.81
CBS328.52	transformant n°23	28.69

[0159] The applicant has made a deposit of some *Myrothecium* strains according to the invention at the BCCM/MUCL Culture Collection (Mycothèque de l'Université Catholique de Louvain, Place Croix du Sud 3, B-1348 LOUVAIN-LA-NEUVE, BELGIUM). Accession numbers are the following: MUCL44828 for the strain *Myrothecium gramineum* CBS449.71, MUCL44829 for the strain *Myrothecium gramineum* IMI140595, MUCL44830 for the strain *Xepiculopsis graminea* (*Myrothecium gramineum*) MUCL39210, MUCL44831 for the strain *Myrothecium verrucaria* CBS328.52 according to the invention. The above deposits under the Budapest Treaty according to Rule 28 EPC were made on 27 august 2003.

CLAIMS

1. A *Myrothecium* host cell comprising at least one recombinant DNA construct for the modulated expression of homologous genes and/or for the expression of
5 heterologous genes.

2. The host cell according to claim 1 characterized in that said the DNA from said construct integrates into the host cell chromosome and/or is present on an episome.

10 3. The host cell according to claim 1 or 2 characterized in that said DNA construct comprises a nucleic acid sequence encoding a heterologous protein and/or homologous protein.

15 4. The host cell according to claim 3 which is a fungal protein.

5. The host cell according to any of the preceding claims characterized in that said DNA construct comprises at least one homologous or heterologous tool that allows or enhances protein expression, said tool being
20 selected from the group consisting of a promoter, a terminator, a polyadenylation signal, a leader, a secretion signal, a selection marker or reporter gene.

25 6. The host cell according to claim 5 characterized in that said homologous or heterologous selection marker gene is selected from the group consisting of a hygromycine B resistance gene, phleomycin resistance gene, a phosphinothricine resistance gene, acetamidase gene, a pyrG gene, an argB gene, a niaD gene and a trpC gene.

30 7. The host cell according to claim 5 characterized in that said homologous or heterologous promoter is selected from the group consisting of the *Aspergillus oryzae* TAKA-amylase promoter, the *Rhizomucor miehei* aspartic proteinase promoter, the *A. niger*

gluocamylase promoter, the *A. niger* neutral α -amylase promoter, the *A. niger* acid stable α -amylase promoter, the *R. miehei* lipase promoter and the promoters of the glycolytic enzymes genes GPD, PGK and ADH.

5 8. The host cell according to claim 5 characterized in that said promoter is regulatable.

 9. The host cell according to any of the preceding claims selected from the group of *Myrothecium* sp. consisting of *Myrothecium inundatum*, *Myrothecium prestonii*,
10 *Myrothecium leucotrichum*, *Myrothecium cinctum*, *Myrothecium masonii*, *Myrothecium roridum*, *Myrothecium verrucaria*, *Myrothecium carmichaelii*, *Myrothecium lachastrae*, *Myrothecium atrum*, *Myrothecium atroviride*, *Myrothecium gramineum* (syn. *Xepiculopsis gramineae*).

15 10. The host cell according to claim 9 characterized in that said host cell is selected from the group consisting of the *Myrothecium gramineum* strain MUCL39210, the *Myrothecium gramineum* strain CBS449.71, the *Myrothecium gramineum* IMI140595, the *Myrothecium gramineum*
20 IMI290405 and the *Myrothecium verrucaria* strain CBS328.52.

 11. The host cells according to according to claim 9 characterized in that said host cell is the *Myrothecium gramineum* strain MUCL39210.

 12. The host cell according to any of the preceding claims comprising a PCNS43 or a p3SR2 vector.
25

 13. The use of a host cell according to any of claims 1 to 12.

 14. The use of a host cell according to any of claims 1 to 12 as cell factory

30 15. The use of a host cell according to any of claims 1 to 12 for industrial enzyme and/or protein production.

 16. The use according to claim 15 wherein said protein and/or enzyme is a therapeutic drug.

17. The use of a host cell according to any of claims 1 to 12 as source of biopesticide.

18. A method of transforming *Myrothecium* sp., said method comprising the steps of:

- 5 - growing *Myrothecium* cells or protoplasts;
- generating a host cell according to any of claims 1 to 12 by introducing into said cells or protoplasts at least one recombinant DNA construct for the modulated expression of homologous genes and/or for the
- 10 expression of heterologous genes into *Myrothecium* cells; and
- selecting genetically modified *Myrothecium* cells.

19. The method according to claim 18 wherein said DNA construct is a plasmid or a vector.

15 20. A transformed *Myrothecium* strain obtainable by a method according to claim 18 or 19.

21. A transformant according to claim 20 with increased amylase activity, increased xylanase activity, increased growth rate, increased biomass production and/or

20 reduced protease production.

22. The transformant according to claim 20 or 21 with an altered metabolic pathway compared to the non-transformed *Myrothecium* strain.

23. A method for producing a protein of

25 interest, said method comprising the steps of:

- culturing *Myrothecium* host cells according to any of claims 1 to 12 under conditions which permit expression of the protein; and
- recovering the protein from said *Myrothecium* culture.

30 24. The method according to claim 23 wherein said protein is a fungal protein.

25. The method according to claim 23 or 24 wherein said protein is an enzyme.

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ccttcgacga gtcgagtagt ttgggaatgc tgctcaaaat gggaggtata tgtcttctaa 240
agctaaatac cggccagaga ccgatagcgc acaagtagag tgatcgaaag atgaaaagca 300
ctttgaaaag agagttaaac agcacgtgaa attgttgaaa gggaagcgtt tatgaccaga 360
cttgggcccgg ttgatcatcc agcgttctcg ctggtgcact ctgccggtcc aggccagcat 420
cagttcgccg cgggggataa aggtttcggg aatgtggctc ctccgggagt gttatagccc 480
gttgcgtaat accctgcggt ggactgaggt ccgcgcttct gcaaggatgc tggcgtaatg 540
gtcatcaacg acccgtcttg aaacacggac caaggagtcg tcttcgtatg cgagtgttcg 600
gggtgaaaac ccctacgcgt aatgaaagtg aacgcaggtg agagcttcgg cgcacatcgc 660
accgatcctg atgttctcgg atggatttga gtaagagcat acggggccgg acccgaaaga 720
aggtgaacta tgcctgtata ggggtgaagcc agaggaaact ctggtggagg ctcgcagcgg 780
ttctgacgtg caaatcgatc gtcaaatatg ggcatggggg cgaaagacta atcgaacctt 840
ctagtagctg gtttccgccg aagtttccct caggatagca gtgttgaact cagttttatg 900
aggtaaagcg aatgattagg gactcggggg cgctatttag cttcatcca ttctcaaact 960
ttaaatatgt aagaagccct tgttacttag ttgaacgtgg gcattcgaat gtatcaacac 1020
tagtgggcca tttttggg 1038

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PURA18WO.ST25

<210> 11
 <211> 1044
 <212> DNA
 <213> Myrothecium

<220>
 <221> misc_feature
 <223> 28s rDNA sequence of the strain IMI29040

<400> 11
 cagggattgc ctcagtaacg gcgagtgaag cggcaacagc tcaaatttga aatctggccc 60
 cccggcccga gttgtaattt gcagaggatg cttttggcaa ggtgccttcc gagttccctg 120
 gaacgggacg ccatagaggg tgagagcccc gtctggtcgg acaccgagcc tctgtaaagc 180
 tccttcgacg agtcgagtag tttgggaatg ctgctcaaaa tgggaggtat atgtcttcta 240
 aagctaaata ccggccagag accgatagcg cacaagtaga gtgatcgaaa gatgaaaagc 300
 actttgaaaa gagagttaaa tagcacgtga aattgttgaa agggaagcgt ttatgaccag 360
 acttggtccc gttgatcatc cagcgttctc gctggtgcac tctgccggtc caggccagca 420
 tcagttcgtc gcgggggata aaggcttcgg gaatgtagct ctcttcgggg agtggttatag 480
 cccgttggtg aataccctgc ggtggactga ggtccgcgct ctgcaaggat gctggcgtaa 540
 tggatcatcac gaccgtcttg aaacacggac caaggagtcg tcttcgtatg cgagtgttcg 600
 ggtgtaaaac ccctacgcgt aatgaaagtg aacgcaggtg agagcttcgg cgcacatcgc 660
 accgatcctg atgttctcgg atggatttga gtaagagcat acggggccgg acccgaaaga 720
 aggtgaacta tgcctgtata ggggtgaagcc agaggaaact ctggtggagg ctcgcagcgg 780
 ttctgacgtg caaatcgatc gtcaaatatg ggcattgggg cgaaagacta atcgaacctt 840
 ctagtagctg gtttccgccg aagtttccct caggatagca gtgttgaaact cagttttatg 900
 aggtaaagcg aatgattagg gactcggggg cgctatttag cttcatcca ttctcaaact 960
 ttaaatatgt aagaagccct tgttgcttaa ttgaacgtgg gcattcgaat gtatcaacac 1020
 tagtggggcca tttttggtaa gcag 1044

<210> 12
 <211> 445
 <212> DNA
 <213> Myrothecium

<220>
 <221> misc_feature
 <223> ITS sequence of the strain MUCL39210

<400> 12
 gcgggaccgc cccggcgccc tcgcgccccg gacccaggcg cccgccggag accccaaact 60
 ctatgtttta ctgtacatct cctctgagtg acacataaac aataaataaa aacttttaac 120
 aacggatctc ttggttctgg catcgatgaa gaacgcagcg aaatgcgata agtaatgtga 180
 attgcagaat tcagtgaatc atcgaatctt tgaacgcaca ttgcgccccg cagtattctg 240

PURA18WO.ST25

gcgggcatgc ctgttcgagc gtcatttcaa ccctcaggcc ccagtgccct ggcgttgggg 300
 atcggcacca gggcgtccgc gcaagcggtc tccccgccgg ccccgaaatc tagtggcggg 360
 ctcgctgtag tcctcctctg cgtagtagca caacctcgca gctggaactc ggcggtggcc 420
 ctgccgttaa acaccccact tctga 445

<210> 13
 <211> 459
 <212> DNA
 <213> Myrothecium

<220>
 <221> misc_feature
 <223> ITS sequence of the strain MUCL11831

<400> 13
 gcgggctcag cccccgcgcc ctcgccggcg ccgggaaaca ggcgcccgcc ggagacccaa 60
 actcaatgtt tttcatgcag tattatctga gtggcaaacg caaaaaataa atcaaaactt 120
 ttaacaacgg atctcttggc tctggcatcg atgaagaacg cagcgaaatg cgataagtaa 180
 tgtgaattgc agaattcagt gaatcatcga atctttgaac gcacattgcg cccgcgagta 240
 ttctcgcggg catgcctgtc cgagcgtcat ttcaaccctc agggcccgcc tgcttgacgg 300
 cgcggcgctg gtgttgggga tcggccctaa accgccgtcc cccaaattca gtggcgggtct 360
 cgctgcagcc tcccctgcgt agtagcaaca ctcgcatgcg gagcgcgggc cggccacgcc 420
 gtaaaacccc cgactttctg aacgttgacc tcggatcag 459

<210> 14
 <211> 423
 <212> DNA
 <213> Myrothecium

<220>
 <221> misc_feature
 <223> ITS sequence of the strain CBS449.71

<400> 14
 gcgggaccgc cccggcgcct tcggggcccg aaccaggcgc ccgccggagg ccccaaactc 60
 ttatgtcttt agtggttttc tcctctgagt gacacataaa caaataaata aaaactttca 120
 acaacggatc tcttggttct ggcacgatg aagaacgcag cgaaatgcga taagtaatgt 180
 gaattgcaga attcagtga tcatcgaatc tttgaacgca cattgcgccc gccagtattc 240
 tggcgggcat gcctgttcga gcgtcatttc aaccctcagg ccccagtgcc ctggtgttgg 300
 ggatcggccc agccttctcg caaggccgcc ggccccgaaa tctagtggcg gtctcgctgt 360
 agtcctcctc tgcgtagtag cacaacctcg cagttggaac gcggcggtgg ccatgccgtt 420
 aaa 423

<210> 15
 <211> 445

PURA18WO.ST25

<212> DNA
 <213> Myrothecium

<220>
 <221> misc_feature
 <223> ITS sequence of the strain IMI140595

<400> 15
 gcgggaccgc cccggcgccc tcgcgcccg gaccagggc cccgccggag accccaaact 60
 ctatgtttta ctgtacatct cctctgagtg acacataaac aataaataaa aactttcaac 120
 aacggatctc ttggttctgg catcgatgaa gaacgcagcg aaatgcgata agtaatgtga 180
 attgcagaat tcagtgaatc atcgaatctt tgaacgcaca ttgccccgc cagtattctg 240
 gcgggcatgc ctgttcgagc gtcatttcaa ccctcaggcc cccagtcctt ggcgttgggg 300
 atcggctcag gggcgaccgc gcaagcgcc gcttcccgc ggccccgaaa tctagtggcg 360
 gtctcgctgt agtcctctc tgcgtagtag cacaacctcg cagctggaac gcggcggtgg 420
 ccctgccgta aaacaccca cttct 445

<210> 16
 <211> 434
 <212> DNA
 <213> Myrothecium

<220>
 <221> misc_feature
 <223> ITS sequence of the strain IMI290405

<400> 16
 gcgggaccgc cccggcgccc tcgggcccgg aaccaggcgc ccgccggagg ccccaaacc 60
 tcatgtcttt agtggttttc tcctctgagt gacacataaa caaataaata aaaactttca 120
 acaacggatc tcttggttct ggcatcgatg aagaacgcag cgaaatgcga taagtaatgt 180
 gaattgcaga attcagtga tcatcgaatc tttgaacgca cattgcgccc gccagtattc 240
 tggcgggcat gcctgttcga gcgtcatttc aaccctcagg ccccgagtc ctggcggttg 300
 ggatcggcag cagggcgta agcccgccg ccccgaaatc tagtggcggt ctgctgtag 360
 tcctcctctg cgtagtagca caacctcgca gttggaacgc ggcggtggcc atgccgttaa 420
 acacccact tctg 434

<210> 17
 <211> 24
 <212> DNA
 <213> Artificial

<220>
 <223> AMY1 primer

<400> 17
 ggaattccac agaaggcatt tatg 24

<210> 18
 <211> 23

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<212> DNA
<213> Artificial

<220>
<223> AMY2 primer

<400> 18
gctctagagc aaccaccagg tca

<210> 19
<211> 17
<212> DNA
<213> Artificial

<220>
<223> Gpd1 primer

<220>
<221> misc_feature
<222> (3)..(3)
<223> n is A, C, T or G

<220>
<221> misc_feature
<222> (12)..(12)
<223> n is dInosine

<400> 19
ggnatcaayg gnttcgg

<210> 20
<211> 20
<212> DNA
<213> Artificial

<220>
<223> Gpd2 primer

<400> 20
gtgswgswgg ggatgatgtt

<210> 21
<211> 23
<212> DNA
<213> Artificial

<220>
<223> Gpd3 primer

<220>
<221> misc_feature
<222> (12)..(12)
<223> n is A, C, T or G

<220>
<221> misc_feature
<222> (18)..(18)
<223> n is dInosine

<400> 21
ggtcgtatcg tnttycgnaa ygc

23

17

20

23

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<210> 22
 <211> 23
 <212> DNA
 <213> Artificial

<220>
 <223> Gpd4 primer

<220>
 <221> misc_feature
 <222> (18)..(18)
 <223> n is dInosine

<400> 22
 ggagccaggc agttggtngt rca

23

<210> 23
 <211> 836
 <212> DNA
 <213> Myrothecium

<220>
 <221> misc_feature
 <223> gpd gene sequence of the strain MUCL11831

<400> 23
 gtggtggtg ggatgatgtt ctgggcagcg ccacggccac cgcgccagtc cttggcggag 60
 ggaccgtcga cggctcttctg ggtggcagtg taggagtgga cgggtgtcat gagaccctca 120
 atgatggtgt acttgctgtt gaggaccttg gcgaggggag ccaggcagtt ggtggtgcac 180
 gaggcgtttg agatgacatc ggcgctgccg tcgtaggtct tctcgttgac gcccatcacg 240
 tacatggggg catcggcgga gggggcggag atgatgacct tcttggcacc gccagcaagg 300
 tgagccttgg ccttgctcgtt ggtggtgaag acaccggttg actcgacaat gtactcggcg 360
 ccggtctcct tccaggggat ggaggcgggg tcacgctcgg tgtagaagcg gaccttcttg 420
 ccgttgacgc tcaggctcagc gccatcggcc tcgacctcac ccttgaagag accgtgggtg 480
 gagtcatact tgagcatgta ggcctagatg gtggtcagct aaagcgctca tttcaagaca 540
 aagaaagcag atgtcaaggt tggcgggaaa agacgatgga ggggcacggg tttggacatg 600
 gttgcaggta ggtggggtgc aacggcccat gtcattgcaa gcatgccatg tcgggttttg 660
 cccctcggat tggatttctt ttttcgcgcc gcatcatgta aagtgggggg aggggcagca 720
 ctcacggcgt acttggtctc gatgaagggg tcgttgacgg cgacaatctc aatgtcgggg 780
 tgctcgacgg cgttgcggaa gacgatacga ccgatgcggc cgaaccatt gatccc 836

<210> 24
 <211> 954
 <212> DNA
 <213> Myrothecium

<220>
 <221> misc_feature
 <223> gpd gene sequence of the strain CBS449.71

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<400> 24
 gtgcagctgg ggatgatgtt ctgggcagca ccacggccac cgcgccagtc cttggcggag 60
 ggaccgtcaa cgggtcttctg ggtggcagtg tagagtggac ggtgggtcatg agaccctcaa 120
 tgatggtgaa cttgtcgttg atgaccttg cgaggggagc caggcagttg gtggtgcaag 180
 aagcgttgga gatgacgtc gcgctgccgt cgtaggtctc ctcgttgaca cccataaacgt 240
 acatgggggc atcggcggag ggagcagaga tgatgacctt cttggcacca cccttcaagt 300
 gagcagcagc cttgtccttg gtggtgaaga caccagtga ctcgacgatg tactcggcgc 360
 cagtctcctt ccaggggatg gcagcggggg cgcgctcagt gtagaagcgg accttcttg 420
 cgttgacagt gaggtcggca ccatcgacgg agacctcacc cttgaagaga ccgtgggtag 480
 agtcatactt gagcatgtag gcctagtga caggggtggt tagcggaatg gccggcagag 540
 agagagtaat tgcggcatga cgaggcgtg gagggaggag cagtcctcgc ccatgacgat 600
 agcattggct attgattcat tcgccgcctt gacagagggc tcgttgaact gcaccaacgc 660
 atgatatcat catggagggg caaatattga cgtgtaatgg tggggtgaat ggcagagtgg 720
 ttgcgttttt tctgccctc acttgagat ggccgtgtcg cgtctgccc tcgtctcct 780
 gcaagtgc at ggtggactgc agctcgttg cctagttgtt ggctgaagg aaacagcact 840
 tacagcgta c ttgggtcaa tgaaggatc gttgacggca acgatctcga cgtcgtcgtg 900
 ctcgacggcg ttgcggaaga cgatacgacc aatgcggccg aaccattga tccc 954

<210> 25
 <211> 1055
 <212> DNA
 <213> Myrothecium

<220>
 <221> misc_feature
 <223> gpd gene sequence of the strain IMI290405

<400> 25
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 ggaccatcga cgggtcttctg ggtggcagtg taggagtga cagtgggtcat gagaccctca 120
 atgatggtga acttgtcgtg aatgacctg gcgagaggag ccaggcagtt ggtgggtgcag 180
 gaggcgttg agatgacgtc ggcgctgccg tcgtaggtct cctcgttgac acccataacg 240
 tacatgggag catcagcaga gggggcagag atgatgacct tcttggcacc acccttcaag 300
 tgagcagcgg ccttgtcctt ggtgggtgaag acaccggtg actcgacgat gtagtcggcg 360
 ccagtctcct tccaaggat ggcagcgggg tcacgctcag ttagaagcg gaccttcttg 420
 ccgttgacgg tcaggtcagc gccatcgacg gtgacctcac cttgaagac gccgtgggtg 480
 gagtcatact tgagcatgta ggcctatgcg tggatggtg tgggaagcat gagtgaattg 540
 gagggattgc gtgaggtga tgaagcatca ttgtggtgtg tcaatggggc tgtttctgct 600
 gctgctggcg gcggttgttg gtggtggtga caaaagaaat ttgttgagcg ggaaaggat 660
 agacggcggc gcatgatatc atggaggggc aaatattgac gcgctgatga tagtggggtg 720

PURA18WO.ST25

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atttttggag gcacctggtt ttgtctttgg ttgcattttt tctgcccctc actcggtcgg      780
tccgtgtctg cggcgcgctg ctgcccctcc tctgtctgca cagagtgcac gctgggctgc      840
agccagctcc gttgcccgtc cgctcgctcg cctgcgtgcc ttgtcccttt ggagctgagg      900
ggaaagaggt gggatcgaga tcacaatcaa aaggttgtag tcacagcgta ggtgggctca      960
atgaagggat cgttgacggc aacgatctcg acgtcggagt gctcgacggc gttgcggaag     1020
acgatacgac caatgcggcc gaaccattg atacc                                  1055

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<210> 26
 <211> 850
 <212> DNA
 <213> Myrothecium

<220>
 <221> misc_feature
 <223> gpd gene sequence of Myrothecium gramineum (Xepiculopsis graminea)
 MUCL39210

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<400> 26
gccgtcgagc actccgacgt cgagatcggt gccgtcaacg accccttcat tgagcccaag      60
tacgtgttaa gtgtgtcttc tgcttcccct cagtcgacga gcgagcccaa agccgagctg     120
cagctagcgg agccatgctg tgcctgcatg cactgcata acagcagcta gaggaggggt     180
acacggccgc gcgcgcagac acacatacaa caccaccacc accaaaagga ggggcagaaa     240
aaatccagca ttgtccgatt tcaccccacc atctcacgtc aaccaatttg cccctccatg     300
atatcatgtg tccgcgcca gctcaacacg tccacctcct ctggccaatg gcgagcgcat     360
tgatgctttg atgagcggaa acgacgctga ggccctcagc ctgctcgtcg ctgccgctgc     420
cgccgcgcgc cgctcacgca tcggcgggct cccgtcgtcg ggcttcaatt gacatgacat     480
gatgcatggc caccgtgcta accaccctg tgtctgtccg ataggcctac atgctcaagt     540
atgactctac ccacggtctc ttcaaggggt aggtcaccgt cgatggcgat gacctgaccg     600
tcaacggcaa gaaggtccgc ttctacactg agcgtgacct cgccgccatc ccctggaagg     660
agactggtgc cgagtacatt gtcgagtcca ccggtgtctt caccaccaag gacaaggctg     720
ctgctcacct gaaggggtgg gccaagaagg tcatcatctc tgccccctct gccgatgccc     780
ccatgtacgt tatgggtgtc aacgaggaga cctacgacgg cagcgccgac gtcattctca     840
acgcttcttg

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<210> 27
 <211> 130
 <212> PRT
 <213> Myrothecium

<220>
 <221> MISC_FEATURE
 <223> glyceraldehyde 3-P dehydrogenase sequence of Myrothecium
 gramineum (Xepiculopsis graminea) MUCL39210

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<400> 27

Ala Val Glu His Ser Asp Val Glu Ile Val Ala Val Asn Asp Pro Phe
 1 5 10 15

Ile Glu Pro Lys Tyr Ala Ala Tyr Met Leu Lys Tyr Asp Ser Thr His
 20 25 30

Gly Leu Phe Lys Gly Glu Val Thr Val Asp Gly Asp Asp Leu Thr Val
 35 40 45

Asn Gly Lys Lys Val Arg Phe Tyr Thr Glu Arg Asp Pro Ala Ala Ile
 50 55 60

Pro Trp Lys Glu Thr Gly Ala Glu Tyr Ile Val Glu Ser Thr Gly Val
 65 70 75 80

Phe Thr Thr Lys Asp Lys Ala Ala Ala His Leu Lys Gly Gly Ala Lys
 85 90 95

Lys Val Ile Ile Ser Ala Pro Ser Ala Asp Ala Pro Met Tyr Val Met
 100 105 110

Gly Val Asn Glu Glu Thr Tyr Asp Gly Ser Ala Asp Val Ile Ser Asn
 115 120 125

Ala Ser
 130

<210> 28
 <211> 21
 <212> DNA
 <213> Artificial

<220>
 <223> hphpCSN431 primer

<400> 28
 atgcctgaac tcaccgcgac g

21

<210> 29
 <211> 18
 <212> DNA
 <213> Artificial

<220>
 <223> hphpCSN432 primer

<400> 29
 ctattccttt gccctcgg

18

<210> 30
 <211> 16
 <212> DNA
 <213> Artificial

<220>

PURA18WO.ST25

<223> GPD2 primer

<400> 30

tctggcatgc ggagag

16

<210> 31

<211> 16

<212> DNA

<213> Artificial

<220>

<223> AMY5 primer

<400> 31

cgatgatgcc ctgcca

16

<210> 32

<211> 850

<212> DNA

<213> Myrothecium

<220>

<221> CDS

<222> (1)..(66)

<220>

<221> misc_feature

<222> (1)..(850)

<223> partial nucleotide sequence and its deduced protein sequence of
the Myrothecium gramineum MUCL39210 glyceraldehyde-3-P
dehydrogenase gene

<220>

<221> CDS

<222> (525)..(848)

<400> 32

gcc gtc gag cac tcc gac gtc gag atc gtt gcc gtc aac gac ccc ttc
Ala Val Glu His Ser Asp Val Glu Ile Val Ala Val Asn Asp Pro Phe
1 5 10 15

48

att gag ccc aag tac gct gtaagtgtg cttctgttc ccctcagtcg
Ile Glu Pro Lys Tyr Ala
20

96

acgagcgagc ccaaagccga gctgcagcta gcggagccat gcgctgcctg catgccactg

156

cataacagca gctagaggag ggggtacacgg ccgcgcgcgc agacacacat acaacaccac

216

caccacaaa aggaggggca gaaaaaatcc agcattgtcc gatttcaccc caccatctca

276

cgtcaaccaa ttgtcccctc catgatata tgtgtccgcg ccagctcaa cacgtccacc

336

tcctctggcc aatggcgagc gcattgatgc ttgtatgagc ggaaacgacg ctgaggccct

396

cagcctcgtc gtcgctgccg ctgccgcgcg gcgccgtca cgcacggcg ggctcccgtc

456

gctgggcttc aattgacatg acatgatgca tggccaccgt gctaaccacc cctgtgtctg

516

tccgatag gcc tac atg ctc aag tat gac tct acc cac ggt ctc ttc aag
Ala Tyr Met Leu Lys Tyr Asp Ser Thr His Gly Leu Phe Lys
25 30 35

566

ggg gag gtc acc gtc gat ggc gat gac ctg acc gtc aac ggc aag aag
Gly Glu Val Thr Val Asp Gly Asp Asp Leu Thr Val Asn Gly Lys Lys

614

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40	45	50	
gtc cgc ttc tac act gag cgt gac ccc gcc gcc atc ccc tgg aag gag Val Arg Phe Tyr Thr Glu Arg Asp Pro Ala Ala Ile Pro Trp Lys Glu 55 60 65			662
act ggt gcc gag tac att gtc gag tcc acc ggt gtc ttc acc acc aag Thr Gly Ala Glu Tyr Ile Val Glu Ser Thr Gly Val Phe Thr Thr Lys 70 75 80			710
gac aag gct gct gct cac ctg aag ggt ggt gcc aag aag gtc atc atc Asp Lys Ala Ala Ala His Leu Lys Gly Gly Ala Lys Lys Val Ile Ile 85 90 95 100			758
tct gcc ccc tct gcc gat gcc ccc atg tac gtt atg ggt gtc aac gag Ser Ala Pro Ser Ala Asp Ala Pro Met Tyr Val Met Gly Val Asn Glu 105 110 115			806
gag acc tac gac ggc agc gcc gac gtc atc tcc aac gct tct tg Glu Thr Tyr Asp Gly Ser Ala Asp Val Ile Ser Asn Ala Ser 120 125 130			850

<210> 33
 <211> 22
 <212> PRT
 <213> Myrothecium

<400> 33

Ala Val Glu His Ser Asp Val Glu Ile Val Ala Val Asn Asp Pro Phe
 1 5 10 15

Ile Glu Pro Lys Tyr Ala
 20

<210> 34
 <211> 108
 <212> PRT
 <213> Myrothecium

<400> 34

Ala Tyr Met Leu Lys Tyr Asp Ser Thr His Gly Leu Phe Lys Gly Glu
 1 5 10 15

Val Thr Val Asp Gly Asp Asp Leu Thr Val Asn Gly Lys Lys Val Arg
 20 25 30

Phe Tyr Thr Glu Arg Asp Pro Ala Ala Ile Pro Trp Lys Glu Thr Gly
 35 40 45

Ala Glu Tyr Ile Val Glu Ser Thr Gly Val Phe Thr Thr Lys Asp Lys
 50 55 60

Ala Ala Ala His Leu Lys Gly Gly Ala Lys Lys Val Ile Ile Ser Ala
 65 70 75 80

Pro Ser Ala Asp Ala Pro Met Tyr Val Met Gly Val Asn Glu Glu Thr
 85 90 95

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Tyr Asp Gly Ser Ala Asp Val Ile Ser Asn Ala Ser
 100 105

<210> 35
 <211> 204
 <212> PRT
 <213> Myrothecium

<220>
 <221> MISC_FEATURE
 <223> partial GPD protein sequence from the strain MUCL39210
 <400> 35

Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Ile Val Phe Arg Asn Ala
 1 5 10 15

Val Glu His Pro Asp Ile Glu Ile Val Ala Val Asn Asp Pro Phe Ile
 20 25 30

Glu Thr Lys Tyr Ala Ala Tyr Met Leu Lys Tyr Asp Ser Thr His Gly
 35 40 45

Leu Phe Lys Gly Glu Val Glu Ala Asp Gly Ala Asp Leu Ser Val Asn
 50 55 60

Gly Lys Lys Val Arg Phe Tyr Thr Glu Arg Asp Pro Ala Ser Ile Pro
 65 70 75 80

Trp Lys Glu Thr Gly Ala Glu Tyr Ile Val Glu Ser Thr Gly Val Phe
 85 90 95

Thr Thr Thr Asp Lys Ala Lys Ala His Leu Ala Gly Gly Ala Lys Lys
 100 105 110

Val Ile Ile Ser Ala Pro Ser Ala Asp Ala Pro Met Tyr Val Met Gly
 115 120 125

Val Asn Glu Lys Thr Tyr Asp Gly Ser Ala Asp Val Ile Ser Asn Ala
 130 135 140

Ser Cys Thr Thr Asn Cys Leu Ala Pro Leu Ala Lys Val Leu Asn Asp
 145 150 155 160

Lys Tyr Thr Ile Ile Glu Gly Leu Met Thr Thr Val His Ser Tyr Thr
 165 170 175

Ala Thr Gln Lys Thr Val Asp Gly Pro Ser Ala Lys Asp Trp Arg Gly
 180 185 190

Gly Arg Gly Ala Ala Gln Asn Ile Ile Pro Thr Thr
 195 200

PURA18WO.ST25

<210> 36
 <211> 204
 <212> PRT
 <213> Myrothecium

<220>
 <221> MISC_FEATURE
 <223> partial GPD protein sequence from the strain MUCL11831

<400> 36

Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Ile Val Phe Arg Asn Ala
 1 5 10 15
 Val Glu His Asp Asp Val Glu Ile Val Ala Val Asn Asp Pro Phe Ile
 20 25 30
 Glu Pro Lys Tyr Ala Ala Tyr Met Leu Lys Tyr Asp Ser Thr His Gly
 35 40 45
 Leu Phe Lys Gly Glu Val Ser Val Asp Gly Ala Asp Leu Thr Val Asn
 50 55 60
 Gly Lys Lys Val Arg Phe Tyr Thr Glu Arg Asp Pro Ala Ala Ile Pro
 65 70 75 80
 Trp Lys Glu Thr Gly Ala Glu Tyr Ile Val Glu Ser Thr Gly Val Phe
 85 90 95
 Thr Thr Lys Asp Lys Ala Ala Ala His Leu Lys Gly Gly Ala Lys Lys
 100 105 110
 Val Ile Ile Ser Ala Pro Ser Ala Asp Ala Pro Met Tyr Val Met Gly
 115 120 125
 Val Asn Glu Glu Thr Tyr Asp Gly Ser Ala Asp Val Ile Ser Asn Ala
 130 135 140
 Ser Cys Thr Thr Asn Cys Leu Ala Pro Leu Ala Lys Val Ile His Asp
 145 150 155 160
 Lys Phe Thr Ile Ile Glu Gly Leu Met Thr Thr Val His Ser Thr Leu
 165 170 175
 Pro Pro Arg Arg Pro Leu Thr Val Pro Pro Pro Arg Thr Gly Ala Val
 180 185 190
 Ala Val Val Leu Pro Arg Thr Ser Ser Pro Ala Ala
 195 200

<210> 37
 <211> 204
 <212> PRT

PURA18WO.ST25

<213> Myrothecium

<220>

<221> MISC_FEATURE

<223> partial GPD protein sequence from the strain CBS449.71

<400> 37

Gly Ile Asn Gly Phe Gly Arg Ile Gly Arg Ile Val Phe Arg Asn Ala
 1 5 10 15

Val Glu His Ser Asp Val Glu Ile Val Ala Val Asn Asp Pro Phe Ile
 20 25 30

Glu Pro Thr Tyr Ala Ala Tyr Met Leu Lys Tyr Asp Ser Thr His Gly
 35 40 45

Val Phe Lys Gly Glu Val Thr Val Asp Gly Ala Asp Leu Thr Val Asn
 50 55 60

Gly Lys Lys Val Arg Phe Tyr Thr Glu Arg Asp Pro Ala Ala Ile Pro
 65 70 75 80

Trp Lys Glu Thr Gly Ala Asp Tyr Ile Val Glu Ser Thr Gly Val Phe
 85 90 95

Thr Thr Lys Asp Lys Ala Ala Ala His Leu Lys Gly Gly Ala Lys Lys
 100 105 110

Val Ile Ile Ser Ala Pro Ser Ala Asp Ala Pro Met Tyr Val Met Gly
 115 120 125

Val Asn Glu Glu Thr Tyr Asp Gly Ser Ala Asp Val Ile Ser Asn Ala
 130 135 140

Ser Cys Thr Thr Asn Cys Leu Ala Pro Leu Ala Lys Val Ile His Asp
 145 150 155 160

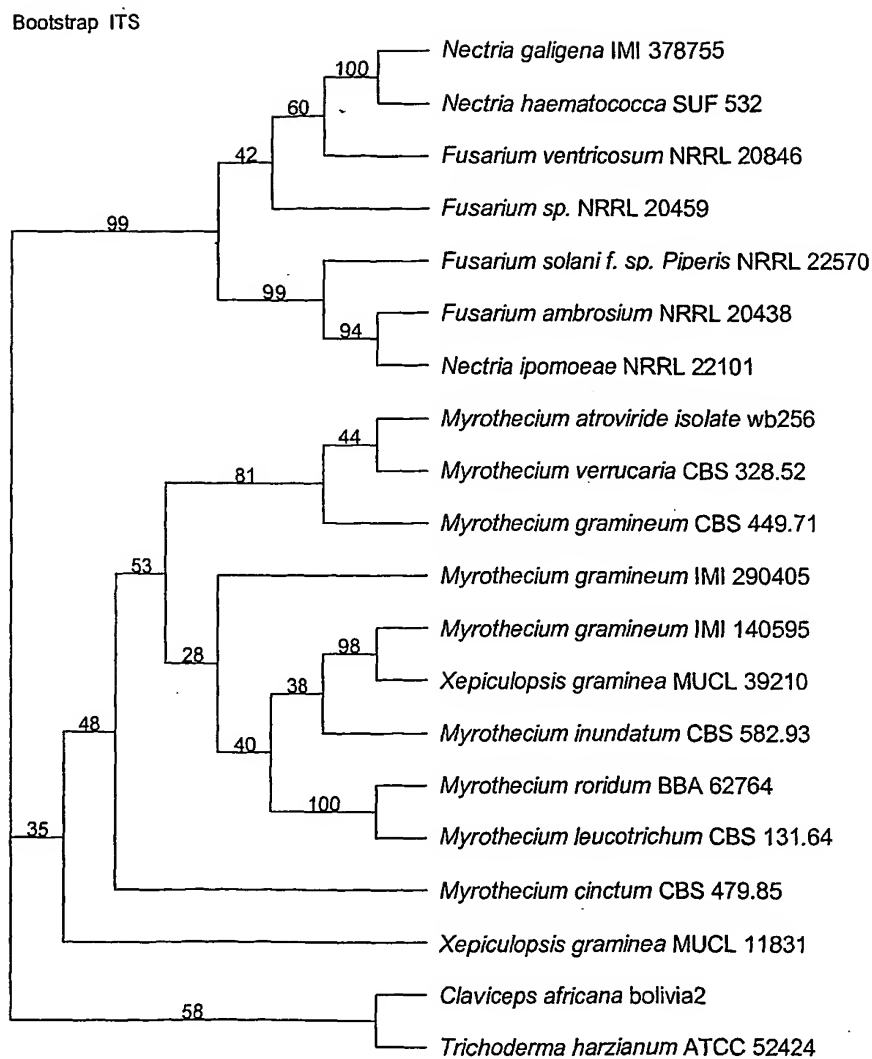
Lys Phe Thr Ile Ile Glu Gly Leu Met Thr Thr Val His Ser Tyr Thr
 165 170 175

Ala Thr Gln Lys Thr Val Asp Gly Pro Ser Ala Lys Asp Trp Arg Gly
 180 185 190

Gly Arg Gly Ala Ala Gln Asn Ile Ile Pro Ser Thr
 195 200

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Fig. 1



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FIG. 2 (SEQ ID NO 32)

5' GCC GTC GAG CAC TCC GAC GTC GAG ATC GTT GCC GTC AAC GAC CCC TTC ATT GAG

A V E H S D V E I V A V N D P F E

63 72 81 90 99 108
CCC AAG TAC GCT GTA AGT GCT GCT TCT GCT TCC CCT CAG TCG ACG AGC GAG CCC

P K Y A (SEQ ID No 33)

117 126 135 144 153 162
AAA GCC GAG CTG CAG CTA GCG GAG CCA TGC GCT GCC TGC ATG CCA CTG CAT AAC

171 180 189 198 207 216
AGC AGC TAG AGG AGG GGT ACA CGG CCG CGC GCG CAG ACA CAC ATA CAA CAC CAC

225 234 243 252 261 270
CAC CAC CAA AAG GAG GGG CAG AAA AAA TCC AGC ATT GTC CGA TTT CAC CCC ACC

279 288 297 306 315 324
ATC TCA CGT CAA CCA ATT TGC CCC TCC ATG ATA TCA TGT GTC CGC GCC CAG CTC

333 342 351 360 369 378
AAC ACG TCC ACC TCC TCT GGC CAA TGG CGA GCG CAT TGA TGC TTT GAT GAG CGG

387 396 405 414 423 432
AAA CGA CGC TGA GGC CCT CAG CCT CGT CGT CGC TGC CGC TGC CGC CGC GCG CCG

441 450 459 468 477 486
CTC ACG CAT CGG CGG GCT CCC GTC GCT GGG CTT CAA TTG ACA TGA CAT GAT GCA

495 504 513 522 531 540
TGG CCA CCG TGC TAA CCA CCC CTG TGT CTG TCC GAT AGG CCT ACA TGC TCA AGT

A Y M L K Y

549 558 567 576 585 594
ATG ACT CTA CCC ACG GTC TCT TCA AGG GTG AGG TCA CCG TCG ATG GCG ATG ACC

D S T H G L F K G E V T V D G D D L

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FIG. 2 (continuation)

603 612 621 630 639 648
TGA CCG TCA ACG GCA AGA AGG TCC GCT TCT ACA CTG AGC GTG ACC CCG CCG CCA

T V N G K K V R F Y T E R D P A A I

657 666 675 684 693 702
TCC CCT GGA AGG AGA CTG GTG CCG AGT ACA TTG TCG AGT CCA CCG GTG TCT TCA

P W K E T G A E Y I V E S T G V F T

711 720 729 738 747 756
CCA CCA AGG ACA AGG CTG CTG CTC ACC TGA AGG GTG GTG CCA AGA AGG TCA TCA

T K D K A A A H L K G G A K K V I I

765 774 783 792 801 810
TCT CTG CCC CCT CTG CCG ATG CCC CCA TGT ACG TTA TGG GTG TCA ACG AGG AGA

S A P S A D A P M Y V M G V N E E T

819 828 837 846
CCT ACG ACG GCA GCG CCG ACG TCA TCT CCA ACG CTT CTT G 3'

Y D G S A D V I S N A S (SEQ ID No 34)

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Fig. 3

10

MUCL11831 GINGFGRIGRIVFRNAVEHPDIEIVAVNDPFIETKYA/AYMLKYDSTHGLFKGEVEADGAD
CBS449.71 GINGFGRIGRIVFRNAVEHDDVEIVAVNDPFIETPKYA/AYMLKYDSTHGLFKGEVSVDGAD
IMI290405 GINGFGRIGRIVFRNAVEHSDVEIVAVNDPFIETPTYA/AYMLKYDSTHGLFKGEVTVDGAD
MUCL39210 -----AVEHSDVEIVAVNDPFIETPKYA/AYMLKYDSTHGLFKGEVTVDGDD
**** *:*****.*** *****:*****.*** *

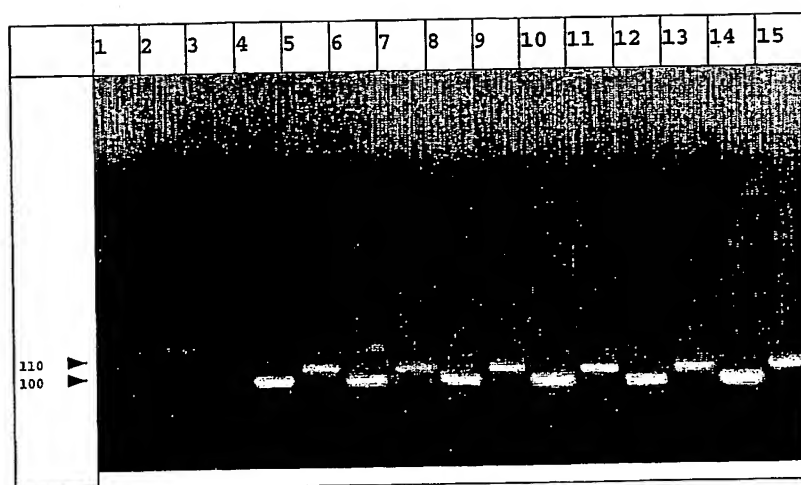
MUCL11831 LSVNGKKVRFYTERDPASIPWKETGAEYIVESTGVFTTTDKAKAHLAGGAKKVIISAPSA
CBS449.71 LTVNGKKVRFYTERDPAAIPWKETGAEYIVESTGVFTTKDAAAHLKGGAKKVIISAPSA
IMI290405 LTVNGKKVRFYTERDPAAIPWKETGADYIVESTGVFTTKDAAAHLKGGAKKVIISAPSA
MUCL39210 LTVNGKKVRFYTERDPAAIPWKETGAEYIVESTGVFTTKDAAAHLKGGAKKVIISAPSA
*:*****:*****:*****.*** ** *****

MUCL11831 DAPMYVMGVNEKTYDGSADVISNASCTTNCLAPLAKVLNDKYTIIIEGLMTTVHSYTATQK
CBS449.71 DAPMYVMGVNEETYDGSADVISNASCTTNCLAPLAKVIHDKFTIIIEGLMTTVHSTLPPRR
IMI290405 DAPMYVMGVNEETYDGSADVISNASCTTNCLAPLAKVIHDKFTIIIEGLMTTVHSYTATQK
MUCL39210 DAPMYVMGVNEETYDGSADVISNAS-----
*****:*****

MUCL11831 TVDGPSAKDWRGGRGAAQNIIPPT (SEQ ID No 35)
CBS449.71 PLTVPPPTGAVAVLPTSSPAA (SEQ ID No 36)
IMI290405 TVDGPSAKDWRGGRGAAQNIIPST (SEQ ID No 37)
MUCL39210 ----- (SEQ ID No 27)

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FIG. 4

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Fig. 5 A

28s rDNA sequence of the strain MUCL39210 (SEQ ID NO 7)

CAGGGATTGCCTCAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGGCC
CCCCGGCCCCGAGTTGTAATTTGCAGAGGATGCTTTTGGCGAGGTGCCTTCCGAGTTCCC
TTGGAACGGGACGCCATAGAGGGTGAGAGCCCCGTCTGGTCGGACACCGAGCCTCTGTAA
AGCTCCTTCGACGAGTCGAGTAGTTTGGGAATGCTGCTCAAAATGGGAGGTATATGTCT
TCTAAAGCTAAATATTGGCCAGAGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGA
AAAGCACTTTGAAGAGAGGTTAAACAGCACGTGAAATTGTTAAAGGGAAGCGTTTAT
GACCAGACTTGGCCCCGGTTGATCATCCAGCGTTCTCGCTGGTGCCTCTGCCGGTCCAG
GCCAGCATCAGTTCGTCGCGGGGGATAAAGTTTTCGGGAATGTGGCTCCTCCGGGAGTG
TTATAGCCCGTTGCGTAATACCCTGCGGTGGACTGAGGTCCGCGCTTCTGCAAGGATGC
TGGCGTAATGGTCATCAACGACCCGTCTTGAAACACGGACCAAGGAGTCGTCTTCGTAT
GCGAGTGTTCCGGGTGTAAAACCCCTACGCGTAATGAAAGTGAACGCAGGTGAGAGCTTC
GGCGCATCATCGACCGATCCTGATGTTCTCGGATGGATTTGAGTAAGAGCATACGGGGC
CGGACCCGAAAGAAGGTGAACTATGCCTGTATAGGGTGAAGCCAGAGGAACTCTGGTG
GAGGCTCGCAGCGGTTCTGACGTGCAAATCGATCGTCAAATATGGGCATGGGGGCGAAA
GACTAATCGAACCTTCTAGTAGCTGGTTTCCGCCGAAGTTCCCTCAGGATAGCAGTGT
TGAACTCAGTTTTATGAGGTAAAGCGAATGATTAGGGACTCGGGGGCGCTATTTAGCCT
TCATCCATTCTCAAACTTTAAATATGTAAGAAGCCCTTGTTACTTAGTTGAACGTGGGC
ATTCGAATGTATCAACACTAGTGGGCCATTTTTGGTAAGCAGAACTGGC

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Fig. 5 B

28s rDNA sequence of the strain MUCL11831 (SEQ ID NO 8)

CAGGGATTGCCTTAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGGAC
CTAGTCCCGAGTTGTAATTTGCAGAGGATGATTTTGGCGCGGTGCCTTCCAAGTTCCCT
GGGACGGGACGCCGGAGAGGGTGAGAGCCCCGTCAGGTTGGACACCAAGCCTATGTAAA
TCTCCTTCGACGAGTCGAGTAGTTTGGGAATGCTGCTCTAAATGGGAGGTATATGTCTT
CTAAAGCTAAATACCGGCCAGAGACCGATAGCGCACAAAGTAGAGTGATCGAAAGATGAA
AAGCACTTTGGAAAGAGAGTTAAACAGCACGTGAAATTGTTAAAAGGGAAGCGTTTACG
ACCAGACTTGTGCCGGTTGATCATCCAGCGTTCTCGCTGGTGCCTCTGCCGGCCCAGG
CCAGCATCAGTTCGCCGCGGGGATAAAGGCGTCGGGAATGTGGCTCCCCCGGGAGTGT
TATAGCCCTTCGCGCAATACCCTGAGGCGGACTGAGGTTTCGCGCATTCGCAAGGATGCT
GGCGTAATGGTCGTCAACGACCCGTCTTGAAACACGGACCAAGGAGTCGTCTTCGTATG
CGAGTGTTTCGGGTGTAAAACCCCTACGCGTAATGAAAGTGAACGCAGGTGAGAGCTTCG
GCGCATCATCGACCGATCCTGATGTTCTCGGATGGATTTGAGTAAGAGCATACGGGGCC
GGACCCGAAAGAAGGTGAACTATGCCTGTATAGGGTGAAGCCAGAGGAACTCTGGTGG
AGGCTCGCAGCGGTTCTGACGTGCAAATCGATCGTCAAATATGGGCATGGGGGCGAAAG
ACTAATCGAACCTTCTAGTAGCTGGTTTCCGCCGAAGTTTCCCTCAGGATAGCAGTGTT
GAACTCAGTTTTATGAGGTAAAGCGAATGATTAGGGACTCGGGGGCGCTATTTAGCCTT
CATCCATTCTCAAACCTTTAAATATGTAAGAAGCCCTTGTTGCTTAATTGAACGTGGGCA
TTCGAATGTATCAACACTAGTGGGCCATTTTTGGTAA

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Fig. 5C

28s rDNA sequence of the strain CBS449.71 (SEQ ID NO 9)

CAGGGATTGCCTCAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGGCC
CTAGGCCCGAGTTGTAATTTGCAGAGGATGCTTTTGGCAAGGTGCCTTCCGAGTTCCCT
GGAACGGGACGCCATAGAGGGTGAGAGCCCCGTCTGGTCGGACACCGAGCCTCTGTAAA
GCTCCTTCGACGAGTCGAGTAGTTTGGGAATGCTGCTCAAAATGGGAGGTATATGTCTT
CTAAAGCTAAATACCGGCCAGAGACCGATAGCGCACAAAGTAGAGTGATCGAAAGATGAA
AAGCACTTTGAAAAGAGAGTTAAATAGCACGTGAAATTGTTGAAAGGGAAGCGTTTATG
ACCAGACTTGGCCCGGTTGATCATCCAGCCTTCTGGCTGGTGCACTCTGCCGGTCCAGG
CCAGCATCAGTTCGTGCGGGGGATAAAGGTTTCGGGAATGTAGCTCCTCCGGGAGTGT
TATAGCCCGTTGCGTAATACCCTGCGGTGGACTGAGGTCCGCGCTCTGCAAGGATGCTG
GCGTAATGGTCATCAACGACCCGTCTTGAAACACGGACCAAGGAGTCGTCTTCGTATGC
GAGTGTTCCGGTGTAACCCCTACGCGTAATGAAAGTGAACGCAGGTGAGAGCTTCGG
CGCATCATCGACCGATCCTGATGTTCTCGGATGGATTTGAGTAAGAGCATACGGGGCCG
GACCCGAAAGAAGGTGAACTATGCCTGTATAGGGTGAAGCCAGAGGAACTCTGGTGGA
GGCTCGCAGCGGTTCTGACGTGCAAATCGATCGTCAAATATGGGCATGGGGGCGAAAGA
CTAATCGAACCTTCTAGTAGCTGGTTTCCGCCGAAGTTTCCCTCAGGATAGCAGTGTTG
AACTCAGTTTTATGAGGTAAAGCGAATGATTAGGGACTCGGGGGCGCTATTTAGCCTTC
ATCCATTCTCAAACCTTTAAATATGTAAGAAGCCCTTGTGCTTAATTGAACGTGGGCAT
TCGAATGTA

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Fig. 5D

28s rDNA sequence of the strain IMI140595 (SEQ ID NO 10)

CAGGGATTGCCTCAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGGCC
CTAGGCCCCGAGTTGTAATTTGCAGAGGATGCTTTTGGCGAGGTGCCTTCCGAGTTCCCT
GGAACGGGACGCCATAGAGGGTGAGAGCCCCGTCTGGTCCGACACCGAGCCTCTGTAAA
GCTCCTTCGACGAGTCGAGTAGTTTGGGAATGCTGCTCAAAATGGGAGGTATATGTCTT
CTAAAGCTAAATACCGGCCAGAGACCGATAGCGCACAAAGTAGAGTGATCGAAAGATGAA
AAGCACTTTGAAAAGAGAGTTAAACAGCACGTGAAATTGTTGAAAGGGAAGCGTTTATG
ACCAGACTTGGCCCGGTTGATCATCCAGCGTTCTCGCTGGTGCCTCTGCCGGTCCAGG
CCAGCATCAGTTCGCCGCGGGGGATAAAGGTTTCGGGAATGTGGCTCCTCCGGGAGTGT
TATAGCCCGTTGCGTAATACCCTGCGGTGGACTGAGGTCCGCGCTTCTGCAAGGATGCT
GGCGTAATGGTCATCAACGACCCGTCTTGAAACACGGACCAAGGAGTCGTCTTCGTATG
CGAGTGTTCCGGTGTA AAAACCCCTACGCGTAATGAAAGTGAACGCAGGTGAGAGCTTCG
GCGCATCATCGACCGATCCTGATGTTCTCGGATGGATTGAGTAAGAGCATACGGGGCC
GGACCCGAAAGAAGGTGAACTATGCCTGTATAGGGTGAAGCCAGAGGAACTCTGGTGG
AGGCTCGCAGCGGTTCTGACGTGCAAATCGATCGTCAAATATGGGCATGGGGGCGAAAG
ACTAATCGAACCTTCTAGTAGCTGGTTTCCGCCGAAGTTTCCCTCAGGATAGCAGTGTT
GAACTCAGTTTTATGAGGTAAAGCGAATGATTAGGGACTCGGGGGCGCTATTTAGCCTT
CATCCATTCTCAAACTTTAAATATGTAAGAAGCCCTTGTTACTTAGTTGAACGTGGGCA
TTCGAATGTATCAACACTAGTGGGCCATTTTTGGT

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Fig. 5E

28s rDNA sequence of the strain IMI290405 (SEQ ID NO 11)

CAGGGATTGCCTCAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGGCC
CCCCGGCCCGAGTTGTAATTTGCAGAGGATGCTTTTGGCAAGGTGCCTTCCGAGTCCC
TGGAACGGGACGCCATAGAGGGTGAGAGCCCCGTCTGGTCGGACACCGAGCCTCTGTAA
AGCTCCTTCGACGAGTCGAGTAGTTTGGGAATGCTGCTCAAAATGGGAGGTATATGTCT
TCTAAAGCTAAATACCGGCCAGAGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGA
AAAGCACTTTGAAAAGAGAGTTAAATAGCACGTGAAATTGTTGAAAGGGAAGCGTTTAT
GACCAGACTTGGCCCGGTTGATCATCCAGCGTTCTCGCTGGTGCACTCTGCCGGTCCAG
GCCAGCATCAGTTCGTCGCGGGGGATAAAGGCTTCGGGAATGTAGCTCTCTTCGGGGAG
TGTTATAGCCCGTTGTGTAATACCCCTGCGGTGGACTGAGGTCCGCGCTCTGCAAGGATG
CTGGCGTAATGGTCATCACGACCGTCTTGAACACGGACCAAGGAGTCGTCTTCGTATG
CGAGTGTTCCGGTGTAACCCCTACGCGTAATGAAAGTGAACGCAGGTGAGAGCTTCG
GCGCATCATCGACCGATCCTGATGTTCTCGGATGGATTTGAGTAAGAGCATACGGGGCC
GGACCCGAAAGAAGGTGAACTATGCCTGTATAGGGTGAAGCCAGAGGAACTCTGGTGG
AGGCTCGCAGCGGTTCTGACGTGCAAATCGATCGTCAAATATGGGCATGGGGGCGAAAG
ACTAATCGAACCTTCTAGTAGCTGGTTTCCGCCGAAGTTCCCTCAGGATAGCAGTGTT
GAACTCAGTTTTATGAGGTAAAGCGAATGATTAGGGACTCGGGGGCGCTATTTAGCCTT
CATCCATTCTCAAACTTTAAATATGTAAGAAGCCCTTGTTGCTTAATTGAACGTGGGCA
TTCGAATGTATCAACACTAGTGGGCCATTTTGGTAAGCAG

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Fig. 6A

ITS sequence of the strain MUCL39210 (SEQ ID NO 12)

GCGGGACGCCCCGGCGCCCTCGCGGCCCCGACCCAGGCGCCCGCCGGAGACCCCAAAC
TCTATGTTTTACTGTACATCTCCTCTGAGTGACACATAAAACAATAAAATAAAAACTTTTA
ACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG
TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTAT
TCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAGGCCCCCAGTGCCTGGCGT
TGGGGATCGGCACCAGGGCGTCCGCGCAAGCGGTCTCCCCGCGGGCCCCGAAATCTAGT
GGCGGTCTCGCTGTAGTCCTCCTCTGCGTAGTAGCACAACTCGCAGCTGGAACCTCGGC
GGTGGCCCTGCCGTTAAACACCCCACTTCTGA

Fig. 6B

ITS sequence of the strain MUCL11831 (SEQ ID NO 13)

GCGGGCTCAGCCCCCGCGCCCTCGCCGGCGCCGGGAAACAGGCGCCCGCCGGAGACCCA
AACTCAATGTTTTTTCATGCAGTATTATCTGAGTGGCAAACGCAAAAAATAAATCAAAAC
TTTTAACAAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAG
TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCG
AGTATTCTCGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCAGGGCCCCGCTGCTT
GCAGGCGCGGCGCTGGTGTGGGGATCGGCCCTAAACCGCCGTCCCCCAAATTCAGTGG
CGGTCTCGCTGCAGCCTCCCCTGCGTAGTAGCAAACTCGCATGCGGAGCGCGGCGCGG
CCACGCCGTAAACCCCCGACTTTCTGAACGTTGACCTCGGATCAG

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Fig. 6C

ITS sequence of the strain CBS449.71 (SEQ ID NO 14)

GCGGGACCGCCCCGGCGCCTTCGGGCCCCGGAACCAGGCGCCCGCCGGAGGCCCCAAACT
CTTATGTCTTTAGTGGTTTTCTCCTCTGAGTGACACATAAACAAATAAATAAAAACTTT
CAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGT
ATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAGGCCCCCAGTGCCTGGT
GTTGGGGATCGGCCCAGCCTTCTCGCAAGGCCGCGGCCCGGAAATCTAGTGGCGGTCT
CGCTGTAGTCCTCCTCTGCGTAGTAGCACAACTCGCAGTTGGAACGCGGCGGTGGCCA
TGCCGTTAAA

Fig. 6D

ITS sequence of the strain IMI140595 (SEQ ID NO 15)

GCGGGACCGCCCCGGCGCCTTCGCGGCCCCGACCCAGGCGCCCGCCGGAGACCCAAAC
TCTATGTTTTACTGTACATCTCCTCTGAGTGACACATAAACAAATAAATAAAAACTTTCA
ACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG
TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTAT
TCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAGGCCCCCAGTGCCTGGCGT
TGGGGATCGGCTCAGGGGCGACCGCGCAAGCGGCCGCTTCCCGCCGGCCCCGAAATCTA
GTGGCGGTCTCGCTGTAGTCCTCCTCTGCGTAGTAGCACAACTCGCAGCTGGAACGCG
GCGGTGGCCCTGCCGTAAAACACCCCACTTCT

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Fig. 6E

ITS sequence of the strain IMI290405 (SEQ ID NO 16)

GCGGGACCGCCCCGGCGCCCTCGGGCCCGGAACCAGGCGCCCGCCGGAGGCCCCAAACC
CTCATGTCTTTAGTGGTTTTCTCCTCTGAGTGACACATAAACAAATAAATAAAAACTTT
CAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA
TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGT
ATTCTGGCGGGCATGCCTGTTCTGAGCGTCATTTCAACCCTCAGGCCCCCAGTGCCTGGC
GTTGGGGATCGGCAGCAGGGCGTCAAGCCCGCCGGCCCCGAAATCTAGTGGCGGTCTCG
CTGTAGTCCTCCTCTGCGTAGTAGCACAACCTCGCAGTTGGAACGCGGCGGTGGCCATG
CCGTTAAACACCCCACTTCTG

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Fig. 7 A

DNA sequence of the gpd gene of the strain MUC11831

GTGGTGGTGGGGATGATGTTCTGGGCAGCGCCACGGCCACCGCGCCAGTCCTTGGCGGA
GGGACCGTCGACGGTCTTCTGGGTGGCAGTGTAGGAGTGGACGGTGGTCATGAGACCCT
CAATGATGGTGTACTTGTCTGTTGAGGACCTTGGCGAGGGGAGCCAGGCAGTTGGTGGTG
CACGAGGCGTTGGAGATGACATCGGCGCTGCCGTCTAGGTCTTCTCGTTGACGCCCAT
CACGTACATGGGGGCATCGGCGGAGGGGGCGGAGATGATGACCTTCTTGGCACCGCCAG
CAAGGTGAGCCTTGGCCTTGTCTGGTGGTGAAGACACCGGTGGACTCGACAATGTAC
TCGGCGCCGGTCTCCTTCCAGGGGATGGAGGCGGGGTACGCTCGGTGTAGAAGCGGAC
CTTCTTGCCGTTGACGCTCAGGTCAGCGCCATCGGCCTCGACCTCACCTTGAAGAGAC
CGTGGGTGGAGTCATACTTGAGCATGTAGGCCTAGATGGTGGTCAGCTAAAGCGCTCAT
TTCAAGACAAAGAAAGCAGATGTCAAGGTTGGCGGGAAGACGATGGAGGGGCACGGG
TTTGGACATGGTTGCAGGTAGGTGGGGTGCAACGGCCCATGTCATTGCAAGCATGCCAT
GTCGGGTTTTGCCCCCTCGGATTGGATTTCTTTTTTCGCGCCGCATCATGTAAAGTGGGG
GGAGGGGCAGCACTCACGGCGTACTTGGTCTCGATGAAGGGGTGTTGACGGCGACAAT
CTCAATGTCGGGGTGCTCGACGGCGTTGCGGAAGACGATACGACCGATGCGGGCCGAACC
CATTGATCCC

(SEQ ID NO 23)

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Fig. 7 B

DNA sequence of the *gpd* gene of the strain CBS449.71

GTGCAGCTGGGGATGATGTTCTGGGCAGCACACGGCCACCGCGCCAGTCCTTGGCGGA
GGGACCGTCAACGGTCTTCTGGGTGGCAGTGTAGAGTGGACGGTGGTCATGAGACCCTC
AATGATGGTGAACTTGTCGTGGATGACCTTGGCGAGGGGAGCCAGGCAGTTGGTGGTG
AAGAAGCGTTGGAGATGACGTCGGCGCTGCCGTCTAGGTCTCCTCGTTGACACCCATA
ACGTACATGGGGGCATCGGCGGAGGGAGCAGAGATGATGACCTTCTTGGCACCACCCTT
CAAGTGAGCAGCAGCCTTGTCCTTGGTGGTGAAGACACCAGTGGACTCGACGATGTACT
CGGCGCCAGTCTCCTTCCAGGGGATGGCAGCGGGGTCGCGCTCAGTGTAGAAGCGGACC
TTCTTGCCGTTGACAGTGAGGTCGGCACCATCGACGGAGACCTCACCTTGAAGAGACC
GTGGGTAGAGTCATACTTGAGCATGTAGGCCTAGTGAACAGGGGTGGTTAGCGGAATGG
CCGGCAGAGAGAGAGTAATTGCGGCATGACGAGGCGTGGGAGGGAGGAGCAGTCCCTCG
CCATGACGATAGCATTTGGCTATTGATTCATTGCGCGCCTTGACAGAGGGCTCGTTGAAC
TGCACCAACGCATGATATCATCATGGAGGGGCAAATATTGACGTGTAATGGTGGGGTGA
ATGGCAGAGTGGTTGCGTTTTTTCTGCCCCCTCACTTGGAGATGGCCGTGTCCGTCTGC
CCCTCGTCTCCCTGCAAGTGCATGGTGGACTGCAGCTCGCTTGCCTAGTTGTTGGCTGA
AGGGAAACAGCACTTACAGCGTACTTGGGCTCAATGAAGGGATCGTTGACGGCAACGAT
CTCGACGTCGTCGTGCTCGACGGCGTTGCGGAAGACGATACGACCAATGCGGCCGAACC
CATTGATCCC

(SEQ ID NO 24)

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Fig. 7C

DNA sequence of the *gpd* gene of the strain IMI290405

GTGGTGGAGGGGATGATGTTCTGGGCAGCACCACGGCCACCACGCCAGTCCTTGGCGGA
GGGACCATCGACGGTCTTCTGGGTGGCAGTGTAGGAGTGGACAGTGGTCATGAGACCCCT
CAATGATGGTGAACCTTGTCGTGAATGACCTTGGCGAGAGGAGCCAGGCAGTTGGTGGTG
CAGGAGGCGTTGGAGATGACGTCGGCGCTGCCGTCGTAGGTCTCCTCGTTGACACCCAT
AACGTACATGGGAGCATCAGCAGAGGGGGCAGAGATGATGACCTTCTTGGCACCACCCT
TCAAGTGAGCAGCGGCCCTTGTCTTGGTGGTGAAGACACCGGTGGACTCGACGATGTAG
TCGGCGCCAGTCTCCTTCCAAGGGATGGCAGCGGGGTACGCTCAGTGTAGAAGCGGAC
CTTCTTGCCGTTGACGGTCAGGTCAGCGCCATCGACGGTGACCTCACCTTGAAGACGC
CGTGGGTGGAGTCATACTTGAGCATGTAGGCCTATGCGTGGATGGTGGTGGGAAGCATG
AGTGAATTGGAGGGATTGCGTGAGGGTGATGAAGCATCATTGTGGTGTGTCAATGGGGC
TGTTTCTGCTGCTGCTGGCGGCGGTTGTTGGTGGTGGTGACAAAAGAAATTTGTTGAGC
GGGAAAGGGATAGACGGCGGCGCATGATATCATGGAGGGGCAAATATTGACGCGCTGAT
GATAGTGGGGTGATTTTTGGAGGCACCTGGTTTTGTCTTTGGTTGCATTTTTTCTGCCC
CTCACTCGGTCCGTCCGTGTCTGCGGCGCGCTCTGCCCCCTCCTCTGTCTGCACAGAGT
GCATGCTGGGCTGCAGCCAGCTCCGTTGCCCCGCTCGCTCGCTCGCCTGCGTGCCTTGTC
CCTTTGGAGCTGAGGGGAAAGAGGTGGGATCGAGATCACAATCAAAAGGTTGTACTCAC
AGCGTAGGTGGGCTCAATGAAGGGATCGTTGACGGCAACGATCTCGACGTCCGAGTGCT
CGACGGCGTTGCGGAAGACGATACGACCAATGCGGCCGAACCCATTGATACC

(SEQ ID NO 25)

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Fig. 8

Sequence of the *gpd* gene of *Myrothecium gramineum*
(*Xepiculopsis graminea*) MUCL39210 (SEQ ID NO 26)

GCCGTCGAGCACTCCGACGTCGAGATCGTTGCCGTCAACGACCCCTTCATTGAGCCCAA
GTACGCTGTAAGTGCTGCTTCTGCTTCCCCCTCAGTCGACGAGCGAGCCCAAAGCCGAGC
TGCAGCTAGCGGAGCCATGCGCTGCCTGCATGCCACTGCATAACAGCAGCTAGAGGAGG
GGTACACGGCCGCGCGCGCAGACACACATAACAACACCACCACCACAAAAGGAGGGGCA
GAAAAAATCCAGCATTTGTCCGATTTACCCCCACCATCTCACGTCAACCAATTTGCCCT
CCATGATATCATGTGTCCGCGCCAGCTCAACACGTCCACCTCCTCTGGCCAATGGCGA
GCGCATTGATGCTTTGATGAGCGGAAACGACGCTGAGGCCCTCAGCCTCGTCGTCGCTG
CCGCTGCCGCCGCGCGCCGCTCACGCATCGGCGGGCTCCCGTCGCTGGGCTTCAATTGA
CATGACATGATGCATGGCCACCGTGCTAACCACCCCTGTGTCTGTCCGATAGGCCTACA
TGCTCAAGTATGACTCTACCCACGGTCTCTTCAAGGGTGAGGTCACCGTCGATGGCGAT
GACCTGACCGTCAACGGCAAGAAGGTCCGCTTCTACACTGAGCGTGACCCCGCCGCCAT
CCCCTGGAAGGAGACTGGTGCCGAGTACATTGTGAGTCCACCGGTGTCTTACCACCA
AGGACAAGGCTGCTGCTCACCTGAAGGGTGGTGCCAAGAAGGTCATCATCTCTGCCCCC
TCTGCCGATGCCCCCATGTACGTTATGGGTGTCAACGAGGAGACCTACGACGGCAGCGC
CGACGTCATCTCCAACGCTTCTTG

Fig. 9

Sequence of the glyceraldehyde 3-P dehydrogenase of
Myrothecium gramineum (*Xepiculopsis graminea*) MUCL39210

AVEHSDVEIVAVNDPFIEPKYAAAYMLKYDSTHGLFKGEVTVDGDDLTVNGKKVRFYTER
DPAAIPWKETGAEYIVESTGVFTTKDKAAAHKGGAKKVIISAPSADAPMYVMGVNEET
YDGSADVISNAS
(SEQ ID NO 27)

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